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(71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US).

(72) Inventors: BARNETT, Susan; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). ZUR MEGEDE, Jan; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). SRIVASTAVA, Indresh; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). LIAN, Ying; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). HARTOG, Karin; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). LIU, Hong; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). GREER, Catherine; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). SELBY, Mark; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US). WALKER, Christopher; Chiron Corporation, 4560 Horton Street - R440, Emeryville, CA 94608 (US).

(74) Agents: DOLLARD, Anne, S.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US) et al.

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(54) Title: IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION OF VIRUS-LIKE PARTICLES

(57) Abstract

The present invention relates to the efficient expression of HIV polypeptides in a variety of cell types, including, but not limited to, mammalian, insect, and plant cells. Synthetic expression cassettes encoding the HIV Gag-containing polypeptides are described, as are uses of the expression cassettes in applications including DNA immunization, generation of packaging cell lines, and production of Env-, tat- or Gag-containing proteins. The invention provides methods of producing Virus-Like Particles (VLPs), as well as, uses of the VLPs including, but not limited to, vehicles for the presentation of antigens and stimulation of immune response in subjects to whom the VLPs are administered.

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IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND
PRODUCTION OF VIRUS-LIKE PARTICLES

5 TECHNICAL FIELD

Synthetic expression cassettes encoding the HIV polypeptides (e.g., Gag-, pol-, prot-, reverse transcriptase, Env- or tat-containing polypeptides) are described, as are uses of the expression cassettes. The present invention relates to the efficient expression of HIV polypeptides in a variety of cell types. Further, the invention provides methods of producing Virus-Like Particles (VLPs), as well as, uses of the VLPs and high level expression of oligomeric envelope proteins.

15

BACKGROUND OF THE INVENTION

Acquired immune deficiency syndrome (AIDS) is recognized as one of the greatest health threats facing modern medicine. There is, as yet, no cure for this disease.

20

In 1983-1984, three groups independently identified the suspected etiological agent of AIDS. See, e.g., Barre-Sinoussi et al. (1983) Science 220:868-871; Montagnier et al., in Human T-Cell Leukemia Viruses (Gallo, Essex & Gross, eds., 1984); Vilmer et al. (1984) The Lancet 1:753; Popovic et al. (1984) Science 224:497-500; Levy et al. (1984) Science 225:840-842. These isolates were variously called lymphadenopathy-associated virus (LAV), human T-cell lymphotropic virus

type III (HTLV-III), or AIDS-associated retrovirus (ARV). All of these isolates are strains of the same virus, and were later collectively named Human Immunodeficiency Virus (HIV). With the isolation of a related

5 AIDS-causing virus, the strains originally called HIV are now termed HIV-1 and the related virus is called HIV-2. See, e.g., Guyader et al. (1987) *Nature* 326:662-669; Brun-Vezinet et al. (1986) *Science* 233:343-346; Clavel et al. (1986) *Nature* 324:691-695.

10 A great deal of information has been gathered about the HIV virus, however, to date an effective vaccine has not been identified. Several targets for vaccine development have been examined including the *env*, *Gag*, *pol* and *tat* gene products encoded by HIV.

15 Haas, et al., (*Current Biology* 6(3):315-324, 1996) suggested that selective codon usage by HIV-1 appeared to account for a substantial fraction of the inefficiency of viral protein synthesis. Andre, et al., (*J. Virol.* 72(2):1497-1503, 1998) described an increased immune
20 response elicited by DNA vaccination employing a synthetic gp120 sequence with optimized codon usage. Schneider, et al., (*J Virol.* 71(7):4892-4903, 1997) discuss inactivation of inhibitory (or instability) elements (INS) located within the coding sequences of the
25 *Gag* and *Gag*-protease coding sequences.

The *Gag* proteins of HIV-1 are necessary for the assembly of virus-like particles. HIV-1 *Gag* proteins are involved in many stages of the life cycle of the virus including, assembly, virion maturation after particle
30 release, and early post-entry steps in virus replication. The roles of HIV-1 *Gag* proteins are numerous and complex (Freed, E.O., *Virology* 251:1-15, 1998).

Wolf, et al., (PCT International Application, WO 96/30523, published 3 October 1996; European Patent Application, Publication No. 0 449 116 A1, published 2 October 1991) have described the use of altered pr55 Gag of HIV-1 to act as a non-infectious retroviral-like particulate carrier, in particular, for the presentation of immunologically important epitopes. Wang, et al., (Virology 200:524-534, 1994) describe a system to study assembly of HIV Gag- β -galactosidase fusion proteins into virions. They describe the construction of sequences encoding HIV Gag- β -galactosidase fusion proteins, the expression of such sequences in the presence of HIV Gag proteins, and assembly of these proteins into virus particles.

Recently, Shiver, et al., (PCT International Application, WO 98/34640, published 13 August 1998) described altering HIV-1 (CAM1) Gag coding sequences to produce synthetic DNA molecules encoding HIV Gag and modifications of HIV Gag. The codons of the synthetic molecules were codons preferred by a projected host cell.

The envelope protein of HIV-1 is a glycoprotein of about 160 kD (gp160). During virus infection of the host cell, gp160 is cleaved by host cell proteases to form gp120 and the integral membrane protein, gp41. The gp41 portion is anchored in (and spans) the membrane bilayer of virion, while the gp120 segment protrudes into the surrounding environment. As there is no covalent attachment between gp120 and gp41, free gp120 is released from the surface of virions and infected cells.

Haas, et al., (Current Biology 6(3):315-324, 1996) suggested that selective codon usage by HIV-1 appeared to account for a substantial fraction of the inefficiency of viral protein synthesis. Andre, et al., (J. Virol.

72(2):1497-1503, 1998) described an increased immune response elicited by DNA vaccination employing a synthetic gp120 sequence with optimized codon usage.

5 **SUMMARY OF THE INVENTION**

The present invention relates to improved expression of HIV Env-, tat-, pol-, prot-, reverse transcriptase, or Gag-containing polypeptides and production of virus-like particles.

10 In one embodiment the present invention includes an expression cassette, comprising a polynucleotide encoding an HIV Gag polypeptide comprising a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:20. In certain embodiments, the polynucleotide
15 sequence encoding said Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:9 or SEQ ID NO:4. The expression cassettes may further include a polynucleotide sequence encoding an HIV protease polypeptide, for
20 example a nucleotide sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:78, and SEQ ID NO:79. The expression cassettes may further include a polynucleotide sequence encoding an HIV reverse
25 transcriptase polypeptide, for example a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, and SEQ ID NO:84. The
30 expression cassettes may further include a polynucleotide sequence encoding an HIV tat polypeptide, for example a sequence selected from the group consisting of: SEQ ID NO:87, SEQ ID NO:88, and SEQ ID NO:89. The expression cassettes may further include a polynucleotide sequence encoding an HIV polymerase polypeptide, for example a

sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6. The expression cassettes may include a polynucleotide sequence encoding an HIV polymerase polypeptide, wherein (i) the nucleotide
5 sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4, and (ii) wherein the sequence is modified by deletions of coding regions corresponding to reverse transcriptase and integrase. The expression
10 cassettes described above may preserve T-helper cell and CTL epitopes. The expression cassettes may further include a polynucleotide sequence encoding an HCV core polypeptide, for example a sequence having at least 90% sequence identity to the sequence presented as SEQ ID
15 NO:7.

In another aspect, the invention includes an expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV Env polypeptide, wherein the polynucleotide sequence encoding said Env
20 polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59). In certain embodiments, the Env expression cassettes include sequences flanking a V1 region but have a deletion in the V1 region itself, for
25 example the sequence presented as SEQ ID NO:65 (Figure 52, gp160.modUS4.delV1). In certain embodiments, the Env expression cassettes include sequences flanking a V2 region but have a deletion in the V2 region itself, for example the sequences shown in SEQ ID NO:60 (Figure 47);
30 SEQ ID NO:66 (Figure 53); SEQ ID NO:34 (Figure 20); SEQ ID NO:37 (Figure 24); SEQ ID NO:40 (Figure 27); SEQ ID NO:43 (Figure 30); SEQ ID NO:46 (Figure 33); SEQ ID NO:76 (Figure 64) and SEQ ID NO:49 (Figure 36). In certain

embodiments, the Env expression cassettes include sequences flanking a V1/V2 region but have a deletion in the V1/V2 region itself, for example, SEQ ID NO:59 (Figure 46); SEQ ID NO:61 (Figure 48); SEQ ID NO:67 (Figure 54); SEQ ID NO:75 (Figure 63); SEQ ID NO:35 (Figure 21); SEQ ID NO:38 (Figure 25); SEQ ID NO:41 (Figure 28); SEQ ID NO:44 (Figure 31); SEQ ID NO:47 (Figure 34) and SEQ ID NO:50 (Figure 37). The Env-encoding expression cassettes may also include a mutated cleavage site that prevents the cleavage of a gp140 polypeptide into a gp120 polypeptide and a gp41 polypeptide, for example, SEQ ID NO:57 (Figure 44); SEQ ID NO:61 (Figure 48); SEQ ID NO:63 (Figure 50); SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34). The Env expression cassettes may include a gp160 Env polypeptide or a polypeptide derived from a gp160 Env polypeptide, for example SEQ ID NO:64 (Figure 51); SEQ ID NO:65 (Figure 52); SEQ ID NO:66 (Figure 53); SEQ ID NO:67 (Figure 54); SEQ ID NO:68 (Figure 55); SEQ ID NO:75 (Figure 63); SEQ ID NO:73 (Figure 61); SEQ ID NO:48 (Figure 35); SEQ ID NO:49 (Figure 36); SEQ ID NO:50 (Figure 37); SEQ ID NO:76 (Figure 64); and SEQ ID NO:74 (Figure 62). The Env expression cassettes may include a gp140 Env polypeptide or a polypeptide derived from a gp140 Env polypeptide, for example SEQ ID NO:56 (Figure 43); SEQ ID NO:57 (Figure 44); SEQ ID NO:58 (Figure 45); SEQ ID NO:59 (Figure 46); SEQ ID NO:60 (Figure 47); SEQ ID NO:61 (Figure 48); SEQ ID NO:62 (Figure 49); SEQ ID NO:63 (Figure 50); SEQ ID NO:36 (Figure 23); SEQ ID NO:37 (Figure 24); SEQ ID NO:38 (Figure 25); SEQ ID NO:39

(Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41
(Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43
(Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45
(Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47
5 (Figure 34). The Env expression cassettes may also
include a gp120 Env polypeptide or a polypeptide derived
from a gp120 Env polypeptide, for example SEQ ID NO:54
(Figure 41); and SEQ ID NO:55 (Figure 42); SEQ ID NO:33
(Figure 19); SEQ ID NO:34 (Figure 20); and SEQ ID NO:35
10 (Figure 21). The Env expression cassettes may include an
Env polypeptide lacking the amino acids corresponding to
residues 128 to about 194, relative to strains SF162 or
US4, for example, SEQ ID NO:55 (Figure 42); SEQ ID NO:62
(Figure 49); SEQ ID NO:63 (Figure 50); and SEQ ID NO:68
15 (Figure 55).

In another aspect, the invention includes a
recombinant expression system for use in a selected host
cell, comprising, one or more of the expression cassettes
described herein operably linked to control elements
20 compatible with expression in the selected host cell. The
expression cassettes may be included on one or on
multiple vectors and may use the same or different
promoters. Exemplary control elements include a
transcription promoter (e.g., CMV, CMV+intron A, SV40,
25 RSV, HIV-Ltr, MMLV-ltr, and metallothionein), a
transcription enhancer element, a transcription
termination signal, polyadenylation sequences, sequences
for optimization of initiation of translation, and
translation termination sequences.

30 In another aspect, the invention includes a
recombinant expression system for use in a selected host
cell, comprising, any one of the expression cassettes
described herein operably linked to control elements

compatible with expression in the selected host cell. Exemplary control elements include, but are not limited to, a transcription promoter (e.g., CMV, CMV+intron A, SV40, RSV, HIV-LTR, MMLV-LTR, and metallothionein), a
5 transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

In yet another aspect, the invention includes a cell
10 comprising one or more of the expression cassettes described herein operably linked to control elements compatible with expression in the cell. The cell can be, for example, a mammalian cell (e.g., BHK, VERO, HT1080, 293, RD, COS-7, or CHO cells), an insect cell (e.g.,
15 *Trichoplusia ni* (Tn5) or Sf9), a bacterial cell, a plant cell, a yeast cell, an antigen presenting cell (e.g., primary, immortalized or tumor-derived lymphoid cells such as macrophages, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof).

20 In another aspect, the invention includes methods for producing a polypeptide including HIV Gag-, prot-, pol-, reverse transcriptase, Env- or Tat-containing polypeptide sequences, said method comprising, incubating the cells comprising one or more the expression cassettes
25 describe herein, under conditions for producing said polypeptide.

In yet another aspect, the invention includes compositions for generating an immunological response, comprising one or more of the expression cassettes
30 described herein. In certain embodiments, the compositions also include an adjuvant.

In a still further aspect, the invention includes methods of generating an immune response in a subject, comprising introducing a composition comprising one or

more of the expression cassettes described herein into the subject under conditions that are compatible with expression of said expression cassette in the subject. In certain embodiments, the expression cassette is introduced using a gene delivery vector. More than one expression cassette may be introduced using one or more gene delivery vectors.

In yet another aspect, the invention includes a purified polynucleotide comprising a polynucleotide sequence encoding a polypeptide including an HIV Env polypeptide, wherein the polynucleotide sequence encoding said Env polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59). Further exemplary purified polynucleotide sequences were presented above.

The polynucleotides of the present invention can be produced by recombinant techniques, synthetic techniques, or combinations thereof.

In another embodiment, the invention includes a method for producing a polypeptide including HIV Gag polypeptide sequences, where the method comprises incubating any of the above cells containing an expression cassette of interest under conditions for producing the polypeptide.

The invention further includes, a method for producing virus-like particles (VLPs) where the method comprises incubating any of the above-described cells containing an expression cassette of interest under conditions for producing VLPs.

In another aspect the invention includes a method for producing a composition of virus-like particles (VLPs) where, any of the above-described cells containing an expression cassette of interest are incubated under

conditions for producing VLPs, and the VLPs are substantially purified to produce a composition of VLPs.

In a further embodiment of the present invention, packaging cell lines are produced using the expression cassettes of the present invention. For example, a cell line useful for packaging lentivirus vectors comprises suitable host cells that have an expression vector containing an expression cassette of the present invention wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell. In a preferred embodiment, such host cells may be transfected with one or more expression cassettes having a polynucleotide sequence that encodes an HIV polymerase polypeptide or polypeptides derived therefrom, for example, where the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6. Further, the HIV polymerase polypeptide may be modified by deletions of coding regions corresponding to reverse transcriptase and integrase. Such a polynucleotide sequence may preserve T-helper cell and CTL epitopes, for example when used in a vaccine application. In addition, the polynucleotide sequence may also include other polypeptides. Further, polynucleotide sequences encoding additional polypeptides whose expression are useful for packaging cell line function may also be utilized.

In another aspect, the present invention includes a gene delivery or vaccine vector for use in a subject, where the vector is a suitable gene delivery vector for use in the subject, and the vector comprises one or more of any of the expression cassettes of the present

invention where the polynucleotide sequences of interest are operably linked to control elements compatible with expression in the subject. Such gene delivery vectors can be used in a method of DNA immunization of a subject, for example, by introducing a gene delivery vector into the subject under conditions that are compatible with expression of the expression cassette in the subject. Gene delivery vectors useful in the practice of the present invention include, but are not limited to, nonviral vectors, bacterial plasmid vectors, viral vectors, particulate carriers (where the vector is coated on a polylactide co-glycolide particles, gold or tungsten particle, for example, the coated particle can be delivered to a subject cell using a gene gun), liposome preparations, and viral vectors (e.g., vectors derived from alphaviruses, pox viruses, and vaccinia viruses, as well as, retroviral vectors, including, but not limited to, lentiviral vectors). Alphavirus-derived vectors include, for example, an alphavirus cDNA construct, a recombinant alphavirus particle preparation and a eukaryotic layered vector initiation system. In one embodiment, the subject is a vertebrate, preferably a mammal, and in a further embodiment the subject is a human.

The invention further includes a method of generating an immune response in a subject, where cells of a subject are transfected with any of the above-described gene delivery vectors (e.g., alphavirus constructs; alphavirus cDNA constructs; eukaryotic layered vector initiation systems (see, e.g., U.S. Patent Number 5,814,482 for description of suitable eukaryotic layered vector initiation systems); alphavirus particle

preparations; etc.) under conditions that permit the expression of a selected polynucleotide and production of a polypeptide of interest (i.e., encoded by any expression cassette of the present invention), thereby
5 eliciting an immunological response to the polypeptide. Transfection of the cells may be performed ex vivo and the transfected cells are reintroduced into the subject. Alternately, or in addition, the cells may be transfected in vivo in the subject. The immune response may be
10 humoral and/or cell-mediated (cellular).

Further embodiments of the present invention include purified polynucleotides. In one embodiment, the purified polynucleotide comprises a polynucleotide sequence having at least 90% sequence identity to the
15 sequence presented as SEQ ID NO:20, and complements thereof. In another embodiment, the purified polynucleotide comprises a polynucleotide sequence encoding an HIV Gag polypeptide, wherein the polynucleotide sequence comprises a sequence having at
20 least 90% sequence identity to the sequence presented as SEQ ID NO:20, and complements thereof. In still another embodiment, the purified polynucleotide comprises a polynucleotide sequence encoding an HIV Gag polypeptide, wherein the polynucleotide sequence comprises a sequence
25 having at least 90% sequence identity to the sequence presented as SEQ ID NO:9, and complements thereof. In further embodiments the polynucleotide sequence comprises a sequence having at least 90% sequence identity to one of the following sequences: SEQ ID NO:4, SEQ ID NO:5, SEQ
30 ID NO:6, SEQ ID NO:7, and complements thereof.

The polynucleotides of the present invention can be produced by recombinant techniques, synthetic techniques, or combinations thereof.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

5 BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the locations of the inactivation sites for the native HIV-1SF2 Gag protein coding sequence.

10 Figure 2 shows the locations of the inactivation sites for the native HIV-1SF2 Gag-protease protein coding sequence.

Figures 3A and 3B show electron micrographs of virus-like particles. Figure 3A shows immature p55Gag virus-like particles in COS-7 cells transfected with a synthetic HIV-1_{SF2} gag construct while Figure 3B shows mature (arrows) and immature VLP in cells transfected with a modified HIV-1_{SF2} gagprotease construct (GP2, SEQ ID NO:70). Transfected cells were fixed at 24 h (gag) or 48 h (gagprotease) post-transfection and subsequently analyzed by electron microscopy (magnification at 100,000X). Cells transfected with vector alone (pCMVKm2) served as negative control (data not shown).

25 Figure 4 presents an image of samples from a series of fractions which were electrophoresed on an 8-16% SDS polyacrylamide gel and the resulting bands visualized by commassie blue staining. The results show that the native p55 Gag virus-like particles (VLPs) banded at a sucrose density of range of 1.15 - 1.19 g/ml with the peak at approximately 1.17 g/ml.

30 Figure 5 presents an image similar to Figure 4 where the analysis was performed using Gag VLPs produced by a synthetic Gag expression cassette.

Figure 6 presents a comparison of the total amount of purified HIV p55 Gag from several preparations obtained from two baculovirus expression cassettes encoding native and modified Gag.

5 Figure 7 presents an alignment of modified coding sequences of the present invention including a synthetic Gag expression cassette (SEQ ID NO:4), a synthetic Gag-protease expression cassette (SEQ ID NO:5), and a synthetic Gag-polymerase expression cassette (SEQ ID
10 NO:6). A common region (Gag-common; SEQ ID NO:9) extends from position 1 to position 1262.

Figure 8 presents an image of wild-type Gag-HCV core expression samples from a series of fractions which were electrophoresed on an 8-16% SDS polyacrylamide gel and
15 the resulting bands visualized by commassie staining.

Figure 9 shows the results of Western blot analysis of the gel shown presented in Figure 8.

Figure 10 presents results similar to those shown in Figure 9. The results in Figure 10 indicate that the
20 main HCV Core-specific reactivity migrates at an approximate molecular weight of 72,000 kD, which is in accordance with the predicted molecular weight of the Gag-HCV core chimeric protein.

Figures 11A to 11D present a comparison of AT
25 content, in percent, of cDNAs corresponding to an unstable human mRNA (human IFN γ mRNA; 11A), wild-type HIV Gag native RNA (11B), a stable human mRNA (human GAPDH mRNA; 11C), and synthetic HIV Gag RNA (11D).

Figure 12 shows the location of the inactivation
30 sites for the native HIV-1SF2 Gag-polymerase sequence.

Figure 13A presents a vector map of pESN2dhfr.

Figure 13B presents a map of the pCMVIII vector.

Figure 14 presents a vector map of pCMV-LINK.

Figure 15 presents a schematic diagram showing the relationships between the following forms of the HIV Env polypeptide: gp160, gp140, gp120, and gp41.

Figure 16 depicts the nucleotide sequence of wild-type gp120 from SF162 (SEQ ID NO:30).

Figure 17 depicts the nucleotide sequence of the wild-type gp140 from SF162 (SEQ ID NO:31).

Figure 18 depicts the nucleotide sequence of the wild-type gp160 from SF162 (SEQ ID NO:32).

Figure 19 depicts the nucleotide sequence of the construct designated gp120.modSF162 (SEQ ID NO:33).

Figure 20 depicts the nucleotide sequence of the construct designated gp120.modSF162.delV2 (SEQ ID NO:34).

Figure 21 depicts the nucleotide sequence of the construct designated gp120.modSF162.delV1/V2 (SEQ ID NO:35).

Figures 22A-H show the percent A-T content over the length of the sequences for IFN γ (Figures 2C and 2G); native gp160 Env US4 and SF162 (Figures 2A and 2E, respectively); GAPDH (Figures 2D and 2H); and the synthetic gp160 Env for US4 and SF162 (Figures 2B and 2F, respectively).

Figure 23 depicts the nucleotide sequence of the construct designated gp140.modSF162 (SEQ ID NO:36).

Figure 24 depicts the nucleotide sequence of the construct designated gp140.modSF162.delV2 (SEQ ID NO:37).

Figure 25 depicts the nucleotide sequence of the construct designated gp140.modSF162.delV1/V2 (SEQ ID NO:38).

Figure 26 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162 (SEQ ID NO:39).

Figure 27 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162.delV2 (SEQ ID NO:40).

Figure 28 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162.delV1/V2 (SEQ ID NO:41).

5 Figure 29 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162 (SEQ ID NO:42).

Figure 30 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162.delV2 (SEQ ID NO:43).

10 Figure 31 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162.delV1/V2 (SEQ ID NO:44).

Figure 32 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162 (SEQ ID NO:45).

15 Figure 33 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162.delV2 (SEQ ID NO:46).

Figure 34 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162.delV1/V2 (SEQ ID NO:47).

20 Figure 35 depicts the nucleotide sequence of the construct designated gp160.modSF162 (SEQ ID NO:48).

Figure 36 depicts the nucleotide sequence of the construct designated gp160.modSF162.delV2 (SEQ ID NO:49).

25 Figure 37 depicts the nucleotide sequence of the construct designated gp160.modSF162.delV1/V2 (SEQ ID NO:50).

Figure 38 depicts the nucleotide sequence of the wild-type gp120 from US4 (SEQ ID NO:51).

30 Figure 39 depicts the nucleotide sequence of the wild-type gp140 from US4 (SEQ ID NO:52).

Figure 40 depicts the nucleotide sequence of the wild-type gp160 from US4 (SEQ ID NO:53).

Figure 41 depicts the nucleotide sequence of the construct designated gp120.modUS4 (SEQ ID NO:54).

Figure 42 depicts the nucleotide sequence of the construct designated gp120.modUS4.del 128-194 (SEQ ID NO:55).

Figure 43 depicts the nucleotide sequence of the construct designated gp140.modUS4 (SEQ ID NO:56).

Figure 44 depicts the nucleotide sequence of the construct designated gp140.mut.modUS4 (SEQ ID NO:57).

Figure 45 depicts the nucleotide sequence of the construct designated gp140.TM.modUS4 (SEQ ID NO:58).

Figure 46 depicts the nucleotide sequence of the construct designated gp140.modUS4.delV1/V2 (SEQ ID NO:59).

Figure 47 depicts the nucleotide sequence of the construct designated gp140.modUS4.delV2 (SEQ ID NO:60).

Figure 48 depicts the nucleotide sequence of the construct designated gp140.mut.modUS4.delV1/V2 (SEQ ID NO:61).

Figure 49 depicts the nucleotide sequence of the construct designated gp140.modUS4.del 128-194 (SEQ ID NO:62).

Figure 50 depicts the nucleotide sequence of the construct designated gp140.mut.modUS4.del 128-194 (SEQ ID NO:63).

Figure 51 depicts the nucleotide sequence of the construct designated gp160.modUS4 (SEQ ID NO:64).

Figure 52 depicts the nucleotide sequence of the construct designated gp160.modUS4.delV1 (SEQ ID NO:65).

Figure 53 depicts the nucleotide sequence of the construct designated gp160.modUS4.delV2 (SEQ ID NO:66).

Figure 54 depicts the nucleotide sequence of the construct designated gp160.modUS4.delV1/V2 (SEQ ID NO:67).

Figure 55 depicts the nucleotide sequence of the construct designated gp160.modUS4.del 128-194 (SEQ ID NO:68).

Figure 56 depicts the nucleotide sequence of the common region of Env from wild-type US4 (SEQ ID NO:69).

Figure 57 depicts the nucleotide sequence of the common region of Env from wild-type SF162 (SEQ ID NO:70).

Figure 58 depicts the nucleotide sequence of synthetic sequences corresponding to the common region of Env from US4 (SEQ ID NO:71).

Figure 59 depicts the nucleotide sequence of synthetic sequences corresponding to the common region of Env from SF162 (SEQ ID NO:72).

Figure 60 presents a schematic representation of an Env polypeptide purification strategy.

Figure 61 depicts the nucleotide sequence of the bicistronic construct designated gp160.modUS4.Gag.modSF2 (SEQ ID NO:73).

Figure 62 depicts the nucleotide sequence of the bicistronic construct designated gp160.modSF162.Gag.modSF2 (SEQ ID NO:74).

Figure 63 depicts the nucleotide sequence of the bicistronic construct designated gp160.modUS4.-delV1/V2.Gag.modSF2 (SEQ ID NO:75).

Figure 64 depicts the nucleotide sequence of the bicistronic construct designated gp160.modSF162.delV2.Gag.modSF2 (SEQ ID NO:76).

Figures 65A-65F show micrographs of 293T cells transfected with the following polypeptide encoding sequences: Figure 65A, gag.modSF2; Figure 65B, gp160.modUS4; Figure 65C, gp160.modUS4.delV1/V2.gag.modSF2 (bicistronic Env and Gag); Figures 65D and 65E, gp160.modUS4.delV1/V2 and

gag.modSF2; and Figure 65F, gp120.modSF162.delV2 and gag.modSF2.

Figures 66A and 66B present alignments of selected modified coding sequences of the present invention including a common region defined for each group of synthetic Env expression cassettes. Figure 66A presents alignments of modified SF162 sequences. Figure 66B presents alignments of modified US4 sequences. The SEQ ID NOs for these sequences are presented in Tables 1A and 1B.

Figure 67 shows the ELISA titers (binding antibodies) obtained in two rhesus macaques (H445, lines with solid black dots; and J408, lines with open squares). The y-axis is the end-point gp140 ELISA titers and the x-axis shows weeks post-immunization. The dashed lines at 0, 4, and 8 weeks represent DNA immunizations. The alternating dash/dotted line at 27 weeks indicates a DNA plus protein boost immunization.

Figure 68 (SEQ ID NO:77) depicts the wild-type nucleotide sequence of Gag reverse transcriptase from SF2.

Figure 69 (SEQ ID NO:78) depicts the nucleotide sequence of the construct designated GP1.

Figure 70 (SEQ ID NO:79) depicts the nucleotide sequence of the construct designated GP2.

Figure 71 (SEQ ID NO:80) depicts the nucleotide sequence of the construct designated FS(+).protinact.RTopt.YM. FS(+) indicates that there is a frameshift in the GagPol coding sequence.

Figure 72 (SEQ ID NO:81) depicts the nucleotide sequence of the construct designated FS(+).protinact.RTopt.YMWM.

Figure 73 (SEQ ID NO:82) depicts the nucleotide sequence of the construct designated FS(-

).protmod.RTopt.YM. FS(-) indicates that there is no frameshift in the GagPol coding sequence.

Figure 74 (SEQ ID NO:83) depicts the nucleotide sequence of the construct designated

5 FS(-).protmod.RTopt.YMWM.

Figure 75 (SEQ ID NO:84) depicts the nucleotide sequence of the construct designated FS(-).protmod.RTopt(+).

Figure 76 (SEQ ID NO:85) depicts the nucleotide
10 sequence of wild type Tat from isolate SF162.

Figure 77 (SEQ ID NO:86) depicts the amino acid sequence of the tat polypeptide.

Figure 78 (SEQ ID NO:87) depicts the nucleotide sequence of a synthetic Tat construct designated
15 Tat.SF162.opt.

Figure 79 (SEQ ID NO:88) depicts the nucleotide sequence of a synthetic Tat construct designated tat.cys22.sf162.opt. The construct encodes a tat polypeptide in which the cystein residue at position 22
20 of the wild type Tat polypeptide is replaced by a glycine residue.

Figures 80A to 80E are an alignment of the nucleotide sequences of the constructs designated Gag.mod.SF2, GP1 (SEQ ID NO:78), and GP2 (SEQ ID NO:79).

25 Figure 81 (SEQ ID NO:89) depicts the nucleotide sequence of the construct designated tataminoSF162.opt, which encodes the amino terminus of that tat protein. The codon encoding the cystein-22 residue is underlined.

Figure 82 (SEQ ID NO:90) depicts the amino acid
30 sequence of the polypeptide encoded by the construct designated tat.cys22.SF162.opt (SEQ ID NO:88).

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel et al. eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream et al., eds., 1998, Academic Press); *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag).

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more such agents.

1. DEFINITIONS

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

"Synthetic" sequences, as used herein, refers to Env-, tat- or Gag-encoding polynucleotides whose expression has been optimized as described herein, for example, by codon substitution, deletions, replacements and/or inactivation of inhibitory sequences. "Wild-type"

or "native" sequences, as used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature, e.g., Gag encoding sequences as found in the isolate HIV-1SF2 or Env encoding sequences as found in the isolates HIV-1SF162 or HIV1US4.

As used herein, the term "virus-like particle" or "VLP" refers to a nonreplicating, viral shell, derived from any of several viruses discussed further below. VLPs are generally composed of one or more viral proteins, such as, but not limited to those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art and discussed more fully below. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, biophysical characterization, and the like. See, e.g., Baker et al., *Biophys. J.* (1991) 60:1445-1456; Hagensee et al., *J. Virol.* (1994) 68:4503-4505. For example, VLPs can be isolated by density gradient centrifugation and/or identified by characteristic density banding (e.g., Example 7). Alternatively, cryoelectron microscopy can be performed on vitrified aqueous samples of the VLP preparation in question, and images recorded under appropriate exposure conditions.

By "particle-forming polypeptide" derived from a particular viral protein is meant a full-length or near full-length viral protein, as well as a fragment thereof, or a viral protein with internal deletions, which has the ability to form VLPs under conditions that favor VLP

formation. Accordingly, the polypeptide may comprise the full-length sequence, fragments, truncated and partial sequences, as well as analogs and precursor forms of the reference molecule. The term therefore intends
5 deletions, additions and substitutions to the sequence, so long as the polypeptide retains the ability to form a VLP. Thus, the term includes natural variations of the specified polypeptide since variations in coat proteins often occur between viral isolates. The term also
10 includes deletions, additions and substitutions that do not naturally occur in the reference protein, so long as the protein retains the ability to form a VLP. Preferred substitutions are those which are conservative in nature, i.e., those substitutions that take place within a family
15 of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline,
20 phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids.

25 An "antigen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is used interchangeably with the term "immunogen."
30 Normally, a B-cell epitope will include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope

will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature), as well as, killed, attenuated or inactivated bacteria, viruses, fungi, parasites or other microbes. Antibodies such as anti-idiotypic antibodies, or fragments thereof, and synthetic peptide mimotopes, which can mimic an antigen or antigenic determinant, are also captured under the definition of antigen as used herein. Similarly, an oligonucleotide or polynucleotide which expresses an antigen or antigenic determinant in vivo, such as in gene therapy and DNA immunization applications, is also included in the definition of antigen herein.

For purposes of the present invention, antigens can be derived from any of several known viruses, bacteria, parasites and fungi, as described more fully below. The term also intends any of the various tumor antigens. Furthermore, for purposes of the present invention, an "antigen" refers to a protein which includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the ability to elicit an immunological response, as defined herein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the antigens.

An "immunological response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. For purposes of the present invention, a "humoral immune response" refers to

an immune response mediated by antibody molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells ("CTL"s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A "cellular immune response" also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+ and CD8+ T-cells.

A composition or vaccine that elicits a cellular immune response may serve to sensitize a vertebrate subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is directed at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host.

The ability of a particular antigen to stimulate a cell-mediated immunological response may be determined by a number of assays, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the

antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., *J. Immunol.* (1993) 151:4189-4199; Doe et al., *Eur. J. Immunol.* (1994) 24:2369-2376. Recent methods of
5 measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of epitope specific T-cells (e.g., by the tetramer technique) (reviewed by McMichael, A.J., and O'Callaghan,
10 C.A., *J. Exp. Med.* 187(9)1367-1371, 1998; Mcheyzer-Williams, M.G., et al, *Immunol. Rev.* 150:5-21, 1996; Lalvani, A., et al, *J. Exp. Med.* 186:859-865, 1997).

Thus, an immunological response as used herein may be one which stimulates the production of CTLs, and/or
15 the production or activation of helper T- cells. The antigen of interest may also elicit an antibody-mediated immune response. Hence, an immunological response may include one or more of the following effects: the production of antibodies by B-cells; and/or the
20 activation of suppressor T-cells and/or $\gamma\delta$ T-cells directed specifically to an antigen or antigens present in the composition or vaccine of interest. These responses may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell
25 cytotoxicity (ADCC) to provide protection to an immunized host. Such responses can be determined using standard immunoassays and neutralization assays, well known in the art.

An "immunogenic composition" is a composition that
30 comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest.

By "subunit vaccine" is meant a vaccine composition which includes one or more selected antigens but not all antigens, derived from or homologous to, an antigen from a pathogen of interest such as from a virus, bacterium, parasite or fungus. Such a composition is substantially free of intact pathogen cells or pathogenic particles, or the lysate of such cells or particles. Thus, a "subunit vaccine" can be prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or analogs thereof. The method of obtaining an antigen included in the subunit vaccine can thus include standard purification techniques, recombinant production, or synthetic production.

"Substantially purified" general refers to isolation of a substance (compound, polynucleotide, protein, polypeptide, polypeptide composition) such that the substance comprises the majority percent of the sample in which it resides. Typically in a sample a substantially purified component comprises 50%, preferably 80%-85%, more preferably 90-95% of the sample. Techniques for purifying polynucleotides and polypeptides of interest are well-known in the art and include, for example, ion-exchange chromatography, affinity chromatography and sedimentation according to density.

A "coding sequence" or a sequence which "encodes" a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control elements"). The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but

is not limited to, cDNA from viral, procaryotic or eucaryotic mRNA, genomic DNA sequences from viral or procaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

Typical "control elements", include, but are not limited to, transcription promoters, transcription enhancer elements, transcription termination signals, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), and translation termination sequences, see e.g., McCaughan et al. (1995) *PNAS USA* 92:5431-5435; Kochetov et al (1998) *FEBS Letts.* 440:351-355.

A "nucleic acid" molecule can include, but is not limited to, procaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between the promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

"Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

Techniques for determining amino acid sequence "similarity" are well known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then

can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

Two or more polynucleotide sequences can be compared by determining their "percent identity." Two or more amino acid sequences likewise can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or peptide sequences, is generally described as the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl. Acids Res.* 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic acid and peptide sequences is provided by the Genetics Computer Group (Madison, WI) in their BestFit utility application. The default parameters for this method are

described in the Wisconsin Sequence Analysis Package
Program Manual, Version 8 (1995) (available from Genetics
Computer Group, Madison, WI). Other equally suitable
programs for calculating the percent identity or
5 similarity between sequences are generally known in the
art.

For example, percent identity of a particular
nucleotide sequence to a reference sequence can be
determined using the homology algorithm of Smith and
10 Waterman with a default scoring table and a gap penalty
of six nucleotide positions. Another method of
establishing percent identity in the context of the
present invention is to use the MPSRCH package of
programs copyrighted by the University of Edinburgh,
15 developed by John F. Collins and Shane S. Sturrok, and
distributed by IntelliGenetics, Inc. (Mountain View, CA).
From this suite of packages, the Smith-Waterman algorithm
can be employed where default parameters are used for the
scoring table (for example, gap open penalty of 12, gap
20 extension penalty of one, and a gap of six). From the
data generated, the "Match" value reflects "sequence
identity." Other suitable programs for calculating the
percent identity or similarity between sequences are
generally known in the art, such as the alignment program
25 BLAST, which can also be used with default parameters.
For example, BLASTN and BLASTP can be used with the
following default parameters: genetic code = standard;
filter = none; strand = both; cutoff = 60; expect = 10;
Matrix = BLOSUM62; Descriptions = 50 sequences; sort by =
30 HIGH SCORE; Databases = non-redundant, GenBank + EMBL +
DDBJ + PDB + GenBank CDS translations + Swiss protein +
Spupdate + PIR. Details of these programs can be found at

the following internet address:

<http://www.ncbi.nlm.gov/cgi-bin/BLAST>.

One of skill in the art can readily determine the proper search parameters to use for a given sequence in the above programs. For example, the search parameters may vary based on the size of the sequence in question. Thus, for example, a representative embodiment of the present invention would include an isolated polynucleotide having X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least about 50% identity to Y contiguous nucleotides derived from any of the sequences described herein, (ii) X equals Y, and (iii) X is greater than or equal to 6 nucleotides and up to 5000 nucleotides, preferably greater than or equal to 8 nucleotides and up to 5000 nucleotides, more preferably 10-12 nucleotides and up to 5000 nucleotides, and even more preferably 15-20 nucleotides, up to the number of nucleotides present in the full-length sequences described herein (e.g., see the Sequence Listing and claims), including all integer values falling within the above-described ranges.

The synthetic expression cassettes (and purified polynucleotides) of the present invention include related polynucleotide sequences having about 80% to 100%, greater than 80-85%, preferably greater than 90-92%, more preferably greater than 95%, and most preferably greater than 98% sequence (including all integer values falling within these described ranges) identity to the synthetic expression cassette sequences disclosed herein (for example, to the sequences presented in Tables 1A and 1B) when the sequences of the present invention are used as the query sequence.

Two nucleic acid fragments are considered to "selectively hybridize" as described herein. The degree of sequence identity between two nucleic acid molecules affects the efficiency and strength of hybridization events between such molecules. A partially identical nucleic acid sequence will at least partially inhibit a completely identical sequence from hybridizing to a target molecule. Inhibition of hybridization of the completely identical sequence can be assessed using hybridization assays that are well known in the art (e.g., Southern blot, Northern blot, solution hybridization, or the like, see Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (1989) Cold Spring Harbor, N.Y.). Such assays can be conducted using varying degrees of selectivity, for example, using conditions varying from low to high stringency. If conditions of low stringency are employed, the absence of non-specific binding can be assessed using a secondary probe that lacks even a partial degree of sequence identity (for example, a probe having less than about 30% sequence identity with the target molecule), such that, in the absence of non-specific binding events, the secondary probe will not hybridize to the target.

When utilizing a hybridization-based detection system, a nucleic acid probe is chosen that is complementary to a target nucleic acid sequence, and then by selection of appropriate conditions the probe and the target sequence "selectively hybridize," or bind, to each other to form a hybrid molecule. A nucleic acid molecule that is capable of hybridizing selectively to a target sequence under "moderately stringent" typically

hybridizes under conditions that allow detection of a target nucleic acid sequence of at least about 10-14 nucleotides in length having at least approximately 70% sequence identity with the sequence of the selected nucleic acid probe. Stringent hybridization conditions typically allow detection of target nucleic acid sequences of at least about 10-14 nucleotides in length having a sequence identity of greater than about 90-95% with the sequence of the selected nucleic acid probe.

Hybridization conditions useful for probe/target hybridization where the probe and target have a specific degree of sequence identity, can be determined as is known in the art (see, for example, Nucleic Acid Hybridization: A Practical Approach, editors B.D. Hames and S.J. Higgins, (1985) Oxford; Washington, DC; IRL Press).

With respect to stringency conditions for hybridization, it is well known in the art that numerous equivalent conditions can be employed to establish a particular stringency by varying, for example, the following factors: the length and nature of probe and target sequences, base composition of the various sequences, concentrations of salts and other hybridization solution components, the presence or absence of blocking agents in the hybridization solutions (e.g., formamide, dextran sulfate, and polyethylene glycol), hybridization reaction temperature and time parameters, as well as, varying wash conditions. The selection of a particular set of hybridization conditions is selected following standard methods in the art (see, for example, Sambrook, et al., Molecular Cloning: A

Laboratory Manual, Second Edition, (1989) Cold Spring Harbor, N.Y.).

5 A first polynucleotide is "derived from" second polynucleotide if it has the same or substantially the same basepair sequence as a region of the second polynucleotide, its cDNA, complements thereof, or if it displays sequence identity as described above.

10 A first polypeptide is "derived from" a second polypeptide if it is (i) encoded by a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described above.

15 Generally, a viral polypeptide is "derived from" a particular polypeptide of a virus (viral polypeptide) if it is (i) encoded by an open reading frame of a polynucleotide of that virus (viral polynucleotide), or (ii) displays sequence identity to polypeptides of that virus as described above.

20 "Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by
25 the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence.

30 "Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for

example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

By "nucleic acid immunization" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal administration, or the like, or can be introduced *ex vivo*, into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

"Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting DNA or RNA of interest into a host cell. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells. Gene delivery expression vectors include, but are not limited to, vectors derived from bacterial plasmid vectors, viral vectors, non-viral vectors, alphaviruses, pox viruses and vaccinia viruses. When used for immunization, such gene delivery expression vectors may be referred to as vaccines or vaccine vectors.

"T lymphocytes" or "T cells" are non-antibody producing lymphocytes that constitute a part of the cell-mediated arm of the immune system. T cells arise from immature lymphocytes that migrate from the bone marrow to

the thymus, where they undergo a maturation process under the direction of thymic hormones. Here, the mature lymphocytes rapidly divide increasing to very large numbers. The maturing T cells become immunocompetent based on their ability to recognize and bind a specific antigen. Activation of immunocompetent T cells is triggered when an antigen binds to the lymphocyte's surface receptors.

The term "transfection" is used to refer to the uptake of foreign DNA by a cell. A cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) *Virology*, 52:456, Sambrook et al. (1989) *Molecular Cloning, a laboratory manual*, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu et al. (1981) *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells. The term refers to both stable and transient uptake of the genetic material, and includes uptake of peptide- or antibody-linked DNAs.

A "vector" is capable of transferring gene sequences to target cells (e.g., bacterial plasmid vectors, viral vectors, non-viral vectors, particulate carriers, and liposomes). Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

Transfer of a "suicide gene" (e.g., a drug-susceptibility gene) to a target cell renders the cell sensitive to compounds or compositions that are

relatively nontoxic to normal cells. Moolten, F.L. (1994) *Cancer Gene Ther.* 1:279-287. Examples of suicide genes are thymidine kinase of herpes simplex virus (HSV-tk), cytochrome P450 (Manome et al. (1996) *Gene Therapy* 3:513-520), human deoxycytidine kinase (Manome et al. (1996) *Nature Medicine* 2(5):567-573) and the bacterial enzyme cytosine deaminase (Dong et al. (1996) *Human Gene Therapy* 7:713-720). Cells which express these genes are rendered sensitive to the effects of the relatively nontoxic prodrugs ganciclovir (HSV-tk), cyclophosphamide (cytochrome P450 2B1), cytosine arabinoside (human deoxycytidine kinase) or 5-fluorocytosine (bacterial cytosine deaminase). Culver et al. (1992) *Science* 256:1550-1552, Huber et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:8302-8306.

A "selectable marker" or "reporter marker" refers to a nucleotide sequence included in a gene transfer vector that has no therapeutic activity, but rather is included to allow for simpler preparation, manufacturing, characterization or testing of the gene transfer vector.

A "specific binding agent" refers to a member of a specific binding pair of molecules wherein one of the molecules specifically binds to the second molecule through chemical and/or physical means. One example of a specific binding agent is an antibody directed against a selected antigen.

By "subject" is meant any member of the subphylum chordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such

as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The term does not denote a particular age. Thus, both adult and newborn individuals are intended to be covered. The system described above
5 is intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material which
10 is not biologically or otherwise undesirable, i.e., the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the
15 composition in which it is contained.

By "physiological pH" or a "pH in the physiological range" is meant a pH in the range of approximately 7.2 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

20 As used herein, "treatment" refers to any of (i) the prevention of infection or reinfection, as in a traditional vaccine, (ii) the reduction or elimination of symptoms, and (iii) the substantial or complete elimination of the pathogen in question. Treatment may
25 be effected prophylactically (prior to infection) or therapeutically (following infection).

"Lentiviral vector", and "recombinant lentiviral vector" are derived from the subset of retroviral vectors known as lentiviruses. Lentiviral vectors refer to a
30 nucleic acid construct which carries, and within certain embodiments, is capable of directing the expression of a nucleic acid molecule of interest. The lentiviral vector includes at least one transcriptional promoter/enhancer or locus defining element(s), or other elements which

control gene expression by other means such as alternate splicing, nuclear RNA export, post-translational modification of messenger, or post-transcriptional modification of protein. Such vector constructs must
5 also include a packaging signal, long terminal repeats (LTRS) or portion thereof, and positive and negative strand primer binding sites appropriate to the lentiviral vector used (if these are not already present in the retroviral vector). Optionally, the recombinant
10 lentiviral vector may also include a signal which directs polyadenylation, selectable markers such as Neo, TK, hygromycin, phleomycin, histidinol, or DHFR, as well as one or more restriction sites and a translation termination sequence. By way of example, such vectors
15 typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis, and a 3' LTR or a portion thereof.

"Lentiviral vector particle" as utilized within the present invention refers to a lentivirus which carries at
20 least one gene of interest. The retrovirus may also contain a selectable marker. The recombinant lentivirus is capable of reverse transcribing its genetic material (RNA) into DNA and incorporating this genetic material into a host cell's DNA upon infection. Lentiviral vector
25 particles may have a lentiviral envelope, a non-lentiviral envelope (e.g., an amphi or VSV-G envelope), or a chimeric envelope.

"Nucleic acid expression vector" or "Expression cassette" refers to an assembly which is capable of
30 directing the expression of a sequence or gene of interest. The nucleic acid expression vector includes a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. Expression cassettes described herein

may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), a multiple cloning site, and a "mammalian" origin of replication (e.g., a SV40 or adenovirus origin of replication).

10 "Packaging cell" refers to a cell which contains those elements necessary for production of infectious recombinant retrovirus (e.g., lentivirus) which are lacking in a recombinant retroviral vector. Typically, such packaging cells contain one or more expression
15 cassettes which are capable of expressing proteins which encode Gag, pol and env proteins.

"Producer cell" or "vector producing cell" refers to a cell which contains all elements necessary for production of recombinant retroviral vector particles.

20

2. MODES OF CARRYING OUT THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such
25 may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar
30 or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

2.1 SYNTHETIC EXPRESSION CASSETTES

2.1.1 MODIFICATION OF HIV-1 GAG NUCLEIC ACID CODING SEQUENCES

One aspect of the present invention is the
5 generation of HIV-1 Gag protein coding sequences, and
related sequences, having improved expression relative to
the corresponding wild-type sequence. An exemplary
embodiment of the present invention is illustrated herein
modifying the Gag protein wild-type sequences obtained
10 from the HIV-1SF2 strain (SEQ ID NO:1; Sanchez-Pescador,
R., et al., Science 227(4686): 484-492, 1985; Luciw,
P.A., et al. U.S. Patent No. 5,156,949, issued October
20, 1992; Luciw, P.A., et al., U.S. Patent No. 5,688,688,
November 18, 1997). Gag sequence obtained from other HIV
15 variants may be manipulated in similar fashion following
the teachings of the present specification. Such other
variants include, but are not limited to, Gag protein
encoding sequences obtained from the isolates HIV_{IIIB},
HIV_{SF2}, HIV-
20 1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4},
other HIV-1 strains from diverse subtypes (e.g.,
subtypes, A through G, and O), HIV-2 strains and diverse
subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}), and simian
immunodeficiency virus (SIV). (See, e.g., Virology, 3rd
25 Edition (W.K. Joklik ed. 1988); Fundamental Virology, 2nd
Edition (B.N. Fields and D.M. Knipe, eds. 1991);
Virology, 3rd Edition (Fields, BN, DM Knipe, PM Howley,
Editors, 1996, Lippincott-Raven, Philadelphia, PA; for a
description of these and other related viruses).

30 First, the HIV-1 codon usage pattern was modified so
that the resulting nucleic acid coding sequence was
comparable to codon usage found in highly expressed human
genes (Example 1). The HIV codon usage reflects a high
content of the nucleotides A or T of the codon-triplet.

The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Gag coding sequences were modified to be comparable to codon usage found in highly expressed human genes. In Figure 11 (Example 1), the percent A-T content of cDNA sequences corresponding to the mRNA for a known unstable mRNA and a known stable mRNA are compared to the percent A-T content of native HIV-1SF2 Gag cDNA and to the synthetic Gag cDNA sequence of the present invention. Experiments performed in support of the present invention showed that the synthetic Gag sequences were capable of higher level of protein production (see the Examples) relative to the native Gag sequences. The data in Figure 11 suggest that one reason for this increased production is increased stability of the mRNA corresponding to the synthetic Gag coding sequences versus the mRNA corresponding to the native Gag coding sequences.

Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of the Gag coding sequences (Example 1). The RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating effects of the INS. To overcome the post-transcriptional activating mechanisms of RRE and Rev, the instability elements were inactivated by introducing multiple point mutations that did not alter the reading frame of the encoded proteins. Figure 1 shows the original SF2 Gag sequence, the location of the INS sequences, and the modifications made to the INS sequences to reduce their effects. The resulting modified coding sequences are

presented as a synthetic Gag expression cassette (SEQ ID NO:4).

Modification of the Gag polypeptide coding sequences resulted in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells). Further, expression of the sequences resulted in production of virus-like particles (VLPs) by these cell lines (see below). Similar Gag polypeptide coding sequences can be obtained from a variety of isolates (families, sub-types, strains, etc.) including, but not limited to such other variants include, but are not limited to, Gag polypeptide encoding sequences obtained from the isolates HIV_{IIIb}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}), and simian immunodeficiency virus (SIV). (See, e.g., Virology, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991; *Virology*, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA). Gag polypeptide encoding sequences derived from these variants can be optimized and tested for improved expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 1).

2.1.2 FURTHER MODIFICATION OF SEQUENCES INCLUDING HIV-1 GAG NUCLEIC ACID CODING SEQUENCES

Experiments performed in support of the present invention have shown that similar modifications of HIV-1 Gag-protease, Gag-reverse transcriptase and Gag-polymerase sequences also result in improved expression

of the polyproteins, as well as, the production of VLPs formed by polypeptides produced from such modified coding sequences.

For the Gag-protease sequence (wild type, SEQ ID NO:2; modified, SEQ ID NOs:5, 78, 79), the changes in codon usage were restricted to the regions upstream of the -1 frameshift (Figure 2). Further, inhibitory (or instability) elements (INS) located within the coding sequences of the Gag-protease polypeptide coding sequence were altered as well (indicated in Figure 2). Exemplary constructs (which include the -1 frameshift) encoding modified Gag-protease sequences include those shown in SEQ ID NOs:78 and 79 (Figures 69 and 70). These are: GP1 (SEQ ID NO:78) in which the protease region was also codon optimized and INS inactivated and GP2 (SEQ ID NO:79), in which the protease region was only subjected to INS inactivation.

For other Gag-containing sequences, for example the Gag-polymerase sequence (wild type, SEQ ID NO:3; modified, SEQ ID NO:6) or Gag-reverse transcriptase (wild type, SEQ ID NO:77; modified SEQ ID NOs:80-84), the changes in codon usage are similar to those for the Gag-protease sequence. Those expression cassettes which contain a frameshift in the GagPol coding sequence are designated "FS(+)" (SEQ ID NOs:80 and 81, Figures 71 and 72) while the designation "FS(-)" (SEQ ID Nos: 82, 83 and 84, Figures 73, 74 and 75) indicates that there is no frameshift utilized in this coding sequence.

In addition to polyproteins containing HIV-related sequences, the various Gag-, Gag-prot, Gag-pol, Gag-reverse transcriptase encoding sequences of the present invention can be fused to other polypeptides (creating chimeric polypeptides) for which an immunogenic response is desired. An example of such a chimeric protein is the

joining of the improved expression Gag encoding sequences to the Hepatitis C Virus (HCV) core protein. In this case, the HCV-core encoding sequences were placed in-frame with the HIV-Gag encoding sequences, resulting in the Gag/HCV-core encoding sequence presented as SEQ ID NO:7 (wild type sequence presented as SEQ ID NO:8).

Further sequences useful in the practice of the present invention include, but are not limited to, sequences encoding viral epitopes/antigens (including but not limited to, HCV antigens (e.g., E1, E2; Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton, M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997), HIV antigens (e.g., derived from *nef*, *tat*, *rev*, *vpu*, *vif*, *vpr* and/or *env*); and sequences encoding tumor antigens/epitopes. Additional sequences are described below. Also, variations on the orientation of the Gag and other coding sequences, relative to each other, are also described below.

Gag, Gag-protease, Gag-reverse transcriptase and/or Gag-polymerase polypeptide coding sequences can be obtained from any HIV isolates (different families, subtypes, and strains) including but not limited to the isolates HIV_{IIIB}, HIV_{SF2}, HIV_{SF162}, HIV_{US4}, HIV_{CM235}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}) (see, e.g., Myers et al. Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico: Los Alamos National Laboratory). Synthetic expression cassettes can be generated using

such coding sequences as starting material by following the teachings of the present specification (e.g., see Example 1). Further, the synthetic expression cassettes of the present invention include related Gag polypeptide coding sequences having greater than 75%, preferably greater than 80-85%, more preferably greater than 90-95%, and most preferably greater than 98% sequence identity (or any integer value within these ranges) to the synthetic expression cassette sequences disclosed herein (for example, SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; and SEQ ID NO:20, the Gag Major Homology Region).

2.1.3 EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1 GAG AND RELATED POLYPEPTIDES

Several synthetic Gag-encoding sequences (expression cassettes) of the present invention were cloned into a number of different expression vectors (Example 1) to evaluate levels of expression and production of VLPs. Two modified synthetic coding sequences are presented as a synthetic Gag expression cassette (SEQ ID NO:4) and a synthetic Gag-protease expression cassette (SEQ ID NOs:78 and 79). Other synthetic Gag-encoding proteins are presented, for example, as SEQ ID NOs:80 through 84. The synthetic DNA fragments for Gag-encoding polypeptides (e.g., Gag, Gag-protease, Gag-polymerase, Gag-reverse transcriptase) were cloned into expression vectors described in Example 1, including, a transient expression vector, CMV-promoter-based mammalian vectors, and a shuttle vector for use in baculovirus expression systems. Corresponding wild-type sequences were cloned into the same vectors.

These vectors were then transfected into a several different cell types, including a variety of mammalian

cell lines, (293, RD, COS-7, and CHO, cell lines available, for example, from the A.T.C.C.). The cell lines were cultured under appropriate conditions and the levels of p24 (Gag) expression in supernatants were
5 evaluated (Example 2). The results of these assays demonstrated that expression of synthetic Gag-encoding sequences were significantly higher than corresponding wild-type sequences (Example 2; Table 2).

Further, Western Blot analysis showed that cells
10 containing the synthetic Gag expression cassette produced the expected 55 kD (p55) protein at higher per-cell concentrations than cells containing the native expression cassette. The Gag p55 protein was seen in both cell lysates and supernatants. The levels of
15 production were significantly higher in cell supernatants for cells transfected with the synthetic Gag expression cassette of the present invention. Experiments performed in support of the present invention suggest that cells containing the synthetic Gag-prot expression cassettes
20 produced the expected Gag-prot protein at comparably higher per-cell concentrations than cells containing the wild-type expression cassette.

Fractionation of the supernatants from mammalian cells transfected with the synthetic Gag expression
25 cassette showed that it provides superior production of both p55 protein and VLPs, relative to the wild-type Gag sequences (Examples 6 and 7).

Efficient expression of these Gag-containing polypeptides in mammalian cell lines provides the
30 following benefits: the Gag polypeptides are free of baculovirus contaminants; production by established methods approved by the FDA; increased purity; greater yields (relative to native coding sequences); and a novel method of producing the Gag-containing polypeptides in

CHO or other mammalian cells which is not feasible in the absence of the increased expression obtained using the constructs of the present invention. Exemplary Mammalian cell lines include, but are not limited to, BHK, VERO, HT1080, 293, 293T, RD, COS-7, CHO, Jurkat, HUT, SUPT, C8166, MOLT4/clone8, MT-2, MT-4, H9, PM1, CEM, myeloma cells (e.g., SB20 cells) and CEMX174, such cell lines are available, for example, from the A.T.C.C.).

A synthetic Gag expression cassette of the present invention also demonstrated high levels of expression and VLP production when transfected into insect cells (Example 7). Further, in addition to a higher total protein yield, the final product from the synthetic p55-expressed Gag consistently contained lower amounts of contaminating baculovirus proteins than the final purified product from the native p55-expressed Gag.

Further, synthetic Gag expression cassettes of the present invention have also been introduced into yeast vectors which were transformed into and efficiently expressed by yeast cells (*Saccharomyces cerevisiae*; using vectors as described in Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998).

In addition to the mammalian and insect vectors described in the Examples, the synthetic expression cassettes of the present invention can be incorporated into a variety of expression vectors using selected expression control elements. Appropriate vectors and control elements for any given cell type can be selected by one having ordinary skill in the art in view of the teachings of the present specification and information known in the art about expression vectors.

For example, a synthetic Gag expression cassette can be inserted into a vector which includes control elements operably linked to the desired coding sequence, which

allow for the expression of the gene in a selected cell-type. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter (a CMV promoter can include intron A), RSV, HIV-LTR, the mouse mammary tumor virus LTR promoter (MMLV-LTR), FIV-LTR, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook, et al., *supra*, as well as a bovine growth hormone terminator sequence. Introns, containing splice donor and acceptor sites, may also be designed into the constructs for use with the present invention (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

The desired synthetic Gag polypeptide encoding sequences can be cloned into any number of commercially available vectors to generate expression of the polypeptide in an appropriate host system. These systems include, but are not limited to, the following:

baculovirus expression {Reilly, P.R., et al., BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL (1992); Beames, et al., Biotechniques 11:378 (1991); Pharmingen; Clontech, Palo Alto, CA)}, vaccinia expression {Earl, P. L., et al., "Expression of proteins in mammalian cells using vaccinia" In Current Protocols in Molecular Biology (F. M. Ausubel, et al. Eds.), Greene Publishing Associates & Wiley Interscience, New York (1991); Moss, B., et al., U.S. Patent Number 5,135,855, issued 4 August 1992}, expression in bacteria {Ausubel, F.M., et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, Inc., Media PA; Clontech}, expression in yeast {Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998; Shuster, J.R., U.S. Patent No. 5,629,203, issued May 13, 1997; Gellissen, G., et al., Antonie Van Leeuwenhoek, 62(1-2):79-93 (1992); Romanos, M.A., et al., Yeast 8(6):423-488 (1992); Goeddel, D.V., Methods in Enzymology 185 (1990); Guthrie, C., and G.R. Fink, Methods in Enzymology 194 (1991)}, expression in mammalian cells {Clontech; Gibco-BRL, Ground Island, NY; e.g., Chinese hamster ovary (CHO) cell lines (Haynes, J., et al., Nuc. Acid. Res. 11:687-706 (1983); 1983, Lau, Y.F., et al., Mol. Cell. Biol. 4:1469-1475 (1984); Kaufman, R. J., "Selection and coamplification of heterologous genes in mammalian cells," in Methods in Enzymology, vol. 185, pp537-566. Academic Press, Inc., San Diego CA (1991)}, and expression in plant cells {plant cloning vectors, Clontech Laboratories, Inc., Palo Alto, CA, and Pharmacia LKB Biotechnology, Inc.,

Piscataway, NJ; Hood, E., et al., *J. Bacteriol.* 168:1291-1301 (1986); Nagel, R., et al., *FEMS Microbiol. Lett.* 67:325 (1990); An, et al., "Binary Vectors", and others in Plant Molecular Biology Manual A3:1-19 (1988);
5 Miki, B.L.A., et al., pp.249-265, and others in Plant DNA Infectious Agents (Hohn, T., et al., eds.) Springer-Verlag, Wien, Austria, (1987); *Plant Molecular Biology: Essential Techniques*, P.G. Jones and J.M. Sutton, New York, J. Wiley, 1997; Miglani, Gurbachan *Dictionary of*
10 *Plant Genetics and Molecular Biology*, New York, Food Products Press, 1998; Henry, R. J., *Practical Applications of Plant Molecular Biology*, New York, Chapman & Hall, 1997}.

Also included in the invention is an expression
15 vector, such as the CMV promoter-containing vectors described in Example 1, containing coding sequences and expression control elements which allow expression of the coding regions in a suitable host. The control elements generally include a promoter, translation initiation
20 codon, and translation and transcription termination sequences, and an insertion site for introducing the insert into the vector. Translational control elements have been reviewed by M. Kozak (e.g., Kozak, M., *Mamm. Genome* 7(8):563-574, 1996; Kozak, M., *Biochimie*
25 76(9):815-821, 1994; Kozak, M., *J Cell Biol* 108(2):229-241, 1989; Kozak, M., and Shatkin, A.J., *Methods Enzymol* 60:360-375, 1979).

Expression in yeast systems has the advantage of commercial production. Recombinant protein production by
30 vaccinia and CHO cell line have the advantage of being mammalian expression systems. Further, vaccinia virus expression has several advantages including the following: (i) its wide host range; (ii) faithful post-

transcriptional modification, processing, folding,
transport, secretion, and assembly of recombinant
proteins; (iii) high level expression of relatively
soluble recombinant proteins; and (iv) a large capacity
5 to accommodate foreign DNA.

The recombinantly expressed polypeptides from
synthetic Gag-encoding expression cassettes are typically
isolated from lysed cells or culture media. Purification
can be carried out by methods known in the art including
10 salt fractionation, ion exchange chromatography, gel
filtration, size-exclusion chromatography, size-
fractionation, and affinity chromatography.

Immunoaffinity chromatography can be employed using
antibodies generated based on, for example, Gag antigens.

15 Advantages of expressing the Gag-containing proteins
of the present invention using mammalian cells include,
but are not limited to, the following: well-established
protocols for scale-up production; the ability to produce
VLPs; cell lines are suitable to meet good manufacturing
20 process (GMP) standards; culture conditions for mammalian
cells are known in the art.

2.1.4 MODIFICATION OF HIV-1 ENV NUCLEIC ACID CODING SEQUENCES

25 One aspect of the present invention is the
generation of HIV-1 Env protein coding sequences, and
related sequences, having improved expression relative to
the corresponding wild-type sequence. Exemplary
embodiments of the present invention are illustrated
30 herein modifying the Env protein wild-type sequences
obtained from the HIV-1 subtype B strains HIV-1US4 and
HIV-1SF162 (Myers et al., Los Alamos Database, Los Alamos
National Laboratory, Los Alamos, New Mexico (1992); Myers
et al., *Human Retroviruses and Aids*, 1997, Los Alamos,

New Mexico: Los Alamos National Laboratory). Env sequence obtained from other HIV variants may be manipulated in similar fashion following the teachings of the present specification. Such other variants include those
5 described above in Section 2.1.1 and on the World Wide Web (Internet), for example at <http://hiv-web.lanl.gov/cgi-bin/hivDB3/public/wdb/ssampublic> and <http://hiv-web.lanl.gov>.

First, the HIV-1 codon usage pattern was modified so
10 that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes (Example 1). The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content
15 in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Env coding sequences were modified to be comparable to codon usage found in highly
20 expressed human genes. Experiments performed in support of the present invention showed that the synthetic Env sequences were capable of higher level of protein production (see the Examples) relative to the native Env sequences. One reason for this increased production may
25 be increased stability of the mRNA corresponding to the synthetic Env coding sequences versus the mRNA corresponding to the native Env coding sequences.

Modification of the Env polypeptide coding sequences resulted in improved expression relative to the wild-type
30 coding sequences in a number of mammalian cell lines. Similar Env polypeptide coding sequences can be obtained from a variety of isolates (families, sub-types, etc.). Env polypeptide encoding sequences derived from these variants can be optimized and tested for improved

expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 2).

5 2.1.5 **FURTHER MODIFICATION OF HIV-1 ENV NUCLEIC ACID
CODING SEQUENCES**

10 In addition to proteins containing HIV-related sequences, the Env encoding sequences of the present invention can be fused to other polypeptides (creating chimeric polypeptides). Also, variations on the orientation of the Env and other coding sequences, relative to each other, are contemplated. Further, the HIV protein encoding cassettes of the present invention can be co-expressed using one vector or multiple vectors. In addition, the polyproteins can be operably linked to the same or different promoters.

20 Env polypeptide coding sequences can be obtained from any HIV isolates (different families, subtypes, and strains) including but not limited to the isolates HIV_{IIIB}, HIV_{SF2}, HIV_{US4}, HIV_{CM235}, HIV_{SF162}, HIV_{LAV}, HIV_{LAI}, HIV_{MN} (see, e.g., Myers et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico: Los Alamos National Laboratory). Synthetic expression cassettes can be generated using such coding sequences as starting material by following the teachings of the present specification (e.g., see Example 1). Further, the synthetic expression cassettes (and purified polynucleotides) of the present invention include related Env polypeptide coding sequences having greater than 90%, preferably greater than 92%, more preferably greater than 95%, and most preferably greater than 98% sequence identity to the synthetic expression cassette sequences disclosed herein (for example, SEQ ID NOs:71-72; and/or

the sequences presented in Tables 1A and 1B) when the sequences of the present invention are used as the query sequence.

5 2.1.6 **EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1
 ENV AND RELATED POLYPEPTIDES**

Several synthetic Env-encoding sequences (expression cassettes) of the present invention were cloned into a number of different expression vectors (Example 1) to
10 evaluate levels of expression and production of Env polypeptide. A modified synthetic coding sequence is presented as synthetic Env expression cassettes (Example 1, e.g., Tables 1A and 1B). The synthetic DNA fragments for Env were cloned into eucaryotic expression vectors
15 described in Example 1 and in Section 2.1.3 above, including, a transient expression vector and CMV-promoter-based mammalian vectors. Corresponding wild-type sequences were cloned into the same vectors.

These vectors were then transfected into a several
20 different cell types, including a variety of mammalian cell lines, (293, RD, COS-7, and CHO, cell lines available, for example, from the A.T.C.C.). The cell lines were cultured under appropriate conditions and the levels of gp120, gp140 and gp160 Env expression in
25 supernatants were evaluated (Example 2). Env polypeptides include, but are not limited to, for example, native gp160, oligomeric gp140, monomeric gp120 as well as modified sequences of these polypeptides. The results of these assays demonstrated that expression of
30 synthetic Env encoding sequences were significantly higher than corresponding wild-type sequences (Example 2; Tables 3 and 4).

Further, Western Blot analysis showed that cells containing the synthetic Env expression cassette produced

the expected protein (gp120, gp140 or gp160) at higher per-cell concentrations than cells containing the native expression cassette. The Env proteins were seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells transfected with the synthetic Env expression cassettes of the present invention as compared to wild type.

Fractionation of the supernatants from mammalian cells transfected with the synthetic Env expression cassettes showed that it provides superior production of Env proteins, relative to the wild-type Env sequences (Examples 2 and 3).

Efficient expression of these Env-containing polypeptides in mammalian cell lines provides the following benefits: the Env polypeptides are free of baculovirus or other viral contaminants; production by established methods approved by the FDA; increased purity; greater yields (relative to native coding sequences); and a novel method of producing the Env-containing polypeptides in CHO cells which is less feasible in the absence of the increased expression obtained using the constructs of the present invention.

Exemplary cell lines (e.g., mammalian, yeast, insect, etc.) include those described above in Section 2.1.3 for Gag-containing constructs. Further, appropriate vectors and control elements (e.g., promoters, enhancers, polyadenylation sequences, etc.) for any given cell type can be selected, as described above in Section 2.1.3, by one having ordinary skill in the art in view of the teachings of the present specification and information known in the art about expression vectors. In addition, the recombinantly expressed polypeptides from synthetic Env-encoding expression cassettes are typically isolated and purified from lysed cells or culture media, as

described above for Gag-encoding expression cassettes. An exemplary purification is described in Example 4 and shown in Figure 60.

5 2.1.7 **MODIFICATION OF HIV-1 TAT NUCLEIC ACID CODING
SEQUENCES**

Another aspect of the present invention is the generation of HIV-1 tat protein coding sequences, and related sequences, having improved expression relative to
10 the corresponding wild-type sequence. Exemplary embodiments of the present invention are illustrated herein modifying the tat wild-type nucleotide sequence (SEQ ID NO:85, Figure 76) obtained from SF162 as described above. Exemplary synthetic tat constructs are
15 shown in SEQ ID NO:87, which depicts a tat construct encoding a full-length tat polypeptide from strain SF162; SEQ ID NO:88, which depicts a tat construct encoding a tat polypeptide having the cystein residue at position 22 changed; and SEQ ID NO:89, which depicts a tat construct
20 encoding the amino terminal portion of a tat polypeptide from strain SF162. The amino portion of the tat protein appears to contain many of the epitopes that induce an immune response. In addition, further modifications include replacement or deletion of the cystein residue at
25 position 22, for example with a valine residue, an alanine residue or a glycine residue (SEQ ID Nos: 88 and 89, Figures 79 and 81), see, e.g., Caputo et al. (1996) *Gene Ther.* 3:235. In Figure 81, which depicts a tat construct encoding the amino terminal portion of a tat
30 polypeptide, the nucleotides (nucleotides 64-66) encoding the cystein residues are underlined. The design and construction of suitable construct can be readily done using

the teachings of the present specification. As with Gag, pol, prot and Env, tat polypeptide coding sequences can be obtained from a variety of isolates (families, subtypes, etc.).

5 Modification of the tat polypeptide coding sequences result in improved expression relative to the wild-type coding sequences in a number of cell lines (e.g., mammalian, yeast, bacterial and insect cells). Tat polypeptide encoding sequences derived from these
10 variants can be optimized and tested for improved expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 2).

 Various forms of the different embodiments of the
15 invention, described herein, may be combined. For example, polynucleotides may be derived from the polynucleotide sequences of the present invention, including, but not limited to, coding sequences for Gag polypeptides, Env polypeptides, polymerase polypeptides,
20 protease polypeptides, tat polypeptides, and reverse transcriptase polypeptides. Further, the polynucleotide coding sequences of the present invention may be combined into multi-cistronic expression cassettes where typically each coding sequence for each polypeptide is preceded by
25 IRES sequences.

2.2 PRODUCTION OF VIRUS-LIKE PARTICLES AND USE OF THE CONSTRUCTS OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES

30 The group-specific antigens (Gag) of human immunodeficiency virus type-1 (HIV-1) self-assemble into noninfectious virus-like particles (VLP) that are released from various eucaryotic cells by budding (reviewed by Freed, E.O., Virology 251:1-15, 1998). The

synthetic expression cassettes of the present invention provide efficient means for the production of HIV-Gag virus-like particles (VLPs) using a variety of different cell types, including, but not limited to, mammalian
5 cells.

Viral particles can be used as a matrix for the proper presentation of an antigen entrapped or associated therewith to the immune system of the host. For example, U.S. Patent No. 4,722,840 describes hybrid particles
10 comprised of a particle-forming fragment of a structural protein from a virus, such as a particle-forming fragment of hepatitis B virus (HBV) surface antigen (HBsAg), fused to a heterologous polypeptide. Tindle et al., *Virology* (1994) 200:547-557, describes the production and use of
15 chimeric HBV core antigen particles containing epitopes of human papillomavirus (HPV) type 16 E7 transforming protein.

Adams et al., *Nature* (1987) 329:68-70, describes the recombinant production of hybrid HIVgpl20:Ty VLPs in
20 yeast and Brown et al., *Virology* (1994) 198:477-488, the production of chimeric proteins consisting of the VP2 protein of human parvovirus B19 and epitopes from human herpes simplex virus type 1, as well as mouse hepatitis virus A59. Wagner et al., (*Virology* (1994) 200:162-175,
25 Brand et al., *J. Virol. Meth.* (1995) 51:153-168; *Virology* (1996) 220:128-140) and Wolf, et al., (EP 0 449 116 A1, published 2 October 1991; WO 96/30523, published 3
October 1996) describe the assembly of chimeric HIV-1 p55Gag particles. U.S. Patent No. 5,503,833 describes
30 the use of rotavirus VP6 spheres for encapsulating and delivering therapeutic agents.

2.2.1 VLP PRODUCTION USING THE SYNTHETIC EXPRESSION
CASSETTES OF THE PRESENT INVENTION

Experiments performed in support of the present invention have demonstrated that the synthetic expression
5 cassettes of the present invention provide superior production of both protein and VLPs, relative to native coding sequences (Examples 7 and 15). Further, electron
microscopic evaluation of VLP production (Examples 6 and 15, Figures 3A-B and 65A-F) showed that free and budding
10 immature virus particles of the expected size were produced by cells containing the synthetic expression cassettes.

Using the synthetic expression cassettes of the present invention, rather than native coding sequences,
15 for the production of virus-like particles provide several advantages. First, VLPs can be produced in enhanced quantity making isolation and purification of the VLPs easier. Second, VLPs can be produced in a variety of cell types using the synthetic expression
20 cassettes, in particular, mammalian cell lines can be used for VLP production, for example, CHO cells. Production using CHO cells provides (i) VLP formation; (ii) correct myristylation and budding; (iii) absence of non-mammalian cell contaminants (e.g., insect viruses
25 and/or cells); and (iv) ease of purification. The synthetic expression cassettes of the present invention are also useful for enhanced expression in cell-types other than mammalian cell lines. For example, infection of insect cells with baculovirus vectors encoding the
30 synthetic expression cassettes resulted in higher levels of total protein yield and higher levels of VLP production (relative to wild-type coding sequences). Further, the final product from insect cells infected with the baculovirus-Gag synthetic expression cassettes

consistently contained lower amounts of contaminating insect proteins than the final product when wild-type coding sequences were used (Examples).

5 VLPs can spontaneously form when the particle-forming polypeptide of interest is recombinantly expressed in an appropriate host cell. Thus, the VLPs produced using the synthetic expression cassettes of the present invention are conveniently prepared using recombinant techniques. As discussed below, the Gag
10 polypeptide encoding synthetic expression cassettes of the present invention can include other polypeptide coding sequences of interest (for example, Env, tat, rev, HIV protease, HIV polymerase, HCV core; see, Example 1). Expression of such synthetic expression cassettes yields
15 VLPs comprising the product of the synthetic expression cassette, as well as, the polypeptide of interest.

Once coding sequences for the desired particle-forming polypeptides have been isolated or synthesized, they can be cloned into any suitable vector or replicon
20 for expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. See, generally, Ausubel et al, *supra* or Sambrook et al, *supra*. The vector is then used to transform an appropriate host
25 cell. Suitable recombinant expression systems include, but are not limited to, bacterial, mammalian, baculovirus/insect, vaccinia, Semliki Forest virus (SFV), Alphaviruses (such as, Sindbis, Venezuelan Equine Encephalitis (VEE)), mammalian, yeast and *Xenopus*
30 expression systems, well known in the art. Particularly preferred expression systems are mammalian cell lines, vaccinia, Sindbis, insect and yeast systems.

For example, a number of mammalian cell lines are known in the art and include immortalized cell lines

available from the American Type Culture Collection (A.T.C.C.), such as, but not limited to, Chinese hamster ovary (CHO) cells, 293 cells, HeLa cells, baby hamster kidney (BHK) cells, mouse myeloma (SB20), monkey kidney cells (COS), as well as others. Similarly, bacterial hosts such as *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*,
10 *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guillerimondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*. See, e.g., Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Fungal hosts include, for example, *Aspergillus*.

20 Viral vectors can be used for the production of particles in eucaryotic cells, such as those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. Additionally, a vaccinia based infection/transfection system, as described in Tomei et
25 al., *J. Virol.* (1993) 67:4017-4026 and Selby et al., *J. Gen. Virol.* (1993) 74:1103-1113, will also find use with the present invention. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This
30 polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus

recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. Alternately, T7 can be added as a purified protein or enzyme as in the "Progenitor" system (Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130). The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation product(s).

Depending on the expression system and host selected, the VLPS are produced by growing host cells transformed by an expression vector under conditions whereby the particle-forming polypeptide is expressed and VLPS can be formed. The selection of the appropriate growth conditions is within the skill of the art. If the VLPS are formed intracellularly, the cells are then disrupted, using chemical, physical or mechanical means, which lyse the cells yet keep the VLPS substantially intact. Such methods are known to those of skill in the art and are described in, e.g., *Protein Purification Applications: A Practical Approach*, (E.L.V. Harris and S. Angal, Eds., 1990).

The particles are then isolated (or substantially purified) using methods that preserve the integrity thereof, such as, by density gradient centrifugation, e.g., sucrose gradients, PEG-precipitation, pelleting, and the like (see, e.g., Kirnbauer et al. *J. Virol.* (1993) 67:6929-6936), as well as standard purification techniques including, e.g., ion exchange and gel filtration chromatography.

VLPS produced by cells containing the synthetic expression cassettes of the present invention can be used to elicit an immune response when administered to a subject. One advantage of the present invention is that VLPS can be produced by mammalian cells carrying the

synthetic expression cassettes at levels previously not possible. As discussed above, the VLPs can comprise a variety of antigens in addition to the Gag polypeptides (e.g., Env, tat, Gag-protease, Gag-polymerase, Gag-HCV-core). Purified VLPs, produced using the synthetic expression cassettes of the present invention, can be administered to a vertebrate subject, usually in the form of vaccine compositions. Combination vaccines may also be used, where such vaccines contain, for example, other subunit proteins derived from HIV or other organisms (e.g., env) or gene delivery vaccines encoding such antigens. Administration can take place using the VLPs formulated alone or formulated with other antigens. Further, the VLPs can be administered prior to, concurrent with, or subsequent to, delivery of the synthetic expression cassettes for DNA immunization (see below) and/or delivery of other vaccines. Also, the site of VLP administration may be the same or different as other vaccine compositions that are being administered. Gene delivery can be accomplished by a number of methods including, but are not limited to, immunization with DNA, alphavirus vectors, pox virus vectors, and vaccinia virus vectors.

VLP immune-stimulating (or vaccine) compositions can include various excipients, adjuvants, carriers, auxiliary substances, modulating agents, and the like. The immune stimulating compositions will include an amount of the VLP/antigen sufficient to mount an immunological response. An appropriate effective amount can be determined by one of skill in the art. Such an amount will fall in a relatively broad range that can be determined through routine trials and will generally be an amount on the order of about 0.1 μ g to about 1000 μ g,

more preferably about 1 μ g to about 300 μ g, of VLP/antigen.

A carrier is optionally present which is a molecule that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycollic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., as well as toxins derived from *E. coli*.

Such adjuvants include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (International Publication No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated

into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA),
(b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below)
5 either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and
(c) Ribit[™] adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group
10 consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox[™]); (3) saponin adjuvants, such as Stimulon[™] (Cambridge Bioscience, Worcester, MA) may be used or particle generated therefrom such as
15 ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, etc.), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), beta chemokines (MIP, 1-
20 alpha, 1-beta Rantes, etc.); (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63)
25 LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109); and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine
30 substituted at position 129) (see, e.g., International Publication Nos. W093/13202 and W092/19265); and (7)

other substances that act as immunostimulating agents to enhance the effectiveness of the composition.

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acteyl-normuramyl-L-alanyl-D-isogluatme (nor-MDP), N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

Dosage treatment with the VLP composition may be a single dose schedule or a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals, chosen to maintain and/or reinforce the immune response, for example at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the potency of the modality, the vaccine delivery employed, the need of the subject and be dependent on the judgment of the practitioner.

If prevention of disease is desired (e.g., reduction of symptoms, recurrences or of disease progression), the antigen carrying VLPs are generally administered prior to primary infection with the pathogen of interest. If treatment is desired, e.g., the reduction of symptoms or recurrences, the VLP compositions are generally administered subsequent to primary infection.

2.2.2 USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES

A number of viral based systems have been developed for use as gene transfer vectors for mammalian host cells. For example, retroviruses (in particular,

lentiviral vectors) provide a convenient platform for gene delivery systems. A coding sequence of interest (for example, a sequence useful for gene therapy applications) can be inserted into a gene delivery vector and packaged in retroviral particles using techniques known in the art. Recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described, including, for example, the following: (U.S. Patent No. 5,219,740; Miller et al. (1989) *Biotechniques* 7:980; Miller, A.D. (1990) *Human Gene Therapy* 1:5; Scarpa et al. (1991) *Virology* 180:849; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033; Boris-Lawrie et al. (1993) *Cur. Opin. Genet. Develop.* 3:102; GB 2200651; EP 0415731; EP 0345242; WO 89/02468; WO 89/05349; WO 89/09271; WO 90/02806; WO 90/07936; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; in U.S. 5,219,740; U.S. 4,405,712; U.S. 4,861,719; U.S. 4,980,289 and U.S. 4,777,127; in U.S. Serial No. 07/800,921; and in Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53:83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci USA* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Sequences useful for gene therapy applications include, but are not limited to, the following. Factor VIII cDNA, including derivatives and deletions thereof (International Publication Nos. WO 96/21035, WO 97/03193, WO 97/03194, WO 97/03195, and WO 97/03191). Factor IX cDNA (Kurachi et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:6461-6464). Factor V cDNA can be obtained from pMT2-V (Jenny (1987) *Proc. Natl. Acad. Sci. USA* 84:4846, A.T.C.C. Deposit No. 40515). A full-length factor V

5 cDNA, or a B domain deletion or B domain substitution thereof, can be used. B domain deletions of factor V, include those reported by Marquette (1995) *Blood* 86:3026 and Kane (1990) *Biochemistry* 29:6762. Antithrombin III
10 cDNA (Prochownik (1983) *J. Biol. Chem.* 258:8389, A.T.C.C. Deposit No. 57224/57225). Protein C encoding cDNA (Foster (1984) *Proc. Natl. Acad. Sci. USA* 81:4766; Beckmann (1985) *Nucleic Acids Res.* 13:5233). Prothrombin cDNA can be obtained by restriction enzyme digestion of a
15 published vector (Degen (1983) *Biochemistry* 22:2087). The endothelial cell surface protein, thrombomodulin, is a necessary cofactor for the normal activation of protein C by thrombin. A soluble recombinant form has been described (Parkinson (1990) *J. Biol. Chem.* 265:12602;
20 Jackman (1987) *Proc. Natl. Acad. Sci. USA* 84:6425; Shirai (1988) *J. Biochem.* 103:281; Wen (1987) *Biochemistry* 26:4350; Suzuki (1987) *EMBO J.* 6:1891, A.T.C.C. Deposit No. 61348, 61349).

20 Many genetic diseases caused by inheritance of defective genes result in the failure to produce normal gene products, for example, thalassemia, phenylketonuria, Lesch-Nyhan syndrome, severe combined immunodeficiency (SCID), hemophilia A and B, cystic fibrosis, Duchenne's Muscular Dystrophy, inherited emphysema and familial
25 hypercholesterolemia (Mulligan et al. (1993) *Science* 260:926; Anderson et al. (1992) *Science* 256:808; Friedman et al. (1989) *Science* 244:1275). Although genetic diseases may result in the absence of a gene product, endocrine disorders, such as diabetes and
30 hypopituitarism, are caused by the inability of the gene to produce adequate levels of the appropriate hormone insulin and human growth hormone respectively.

In one aspect, gene therapy employing the constructs and methods of the present invention involves the

introduction of normal recombinant genes into T cells so that new or missing proteins are produced by the T cells after introduction or reintroduction thereof into a patient. A number of genetic diseases have been selected
5 for treatment with gene therapy, including adenine deaminase deficiency, cystic fibrosis, α_1 -antitrypsin deficiency, Gaucher's syndrome, as well as non-genetic diseases.

In particular, Gaucher's syndrome is a genetic
10 disorder characterized by a deficiency of the enzyme glucocerebrosidase. This enzyme deficiency leads to the accumulation of glucocerebroside in the lysosomes of all cells in the body. For a review see *Science* 256:794 (1992) and Scriver et al., *The Metabolic Basis of*
15 *Inherited Disease*, 6th ed., vol. 2, page 1677). Thus, gene transfer vectors that express glucocerebrosidase can be constructed for use in the treatment of this disorder. Likewise, gene transfer vectors encoding lactase can be used in the treatment of hereditary lactose intolerance,
20 those expressing AD can be used for treatment of ADA deficiency, and gene transfer vectors encoding α_1 -antitrypsin can be used to treat α_1 -antitrypsin deficiency. See Ledley, F.D. (1987) *J. Pediatrics* 110:157-174, Verma, I. (Nov. 1987) *Scientific American*
25 pp. 68-84, and International Publication No. WO 95/27512 entitled "Gene Therapy Treatment for a Variety of Diseases and Disorders," for a description of gene therapy treatment of genetic diseases.

In still further embodiments of the invention,
30 nucleotide sequences which can be incorporated into a gene transfer vector include, but are not limited to, proteins associated with enzyme-deficiency disorders, such as the cystic fibrosis transmembrane regulator (see, for example, U.S. Patent No. 5,240,846 and Larrick et al.

(1991) *Gene Therapy Applications of Molecular Biology*, Elsevier, New York and adenosine deaminase (ADA) (see U.S. Patent No. 5,399,346); growth factors, or an agonist or antagonist of a growth factor (Bandara et al. (1992) *DNA and Cell Biology*, 11:227); one or more tumor suppressor genes such as p53, Rb, or C-CAM1 (Kleiner et al. (1995) *Cancer Research* 55:2831); a molecule that modulates the immune system of an organism, such as a HLA molecule (Nabel et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:11307); a ribozyme (Larsson et al. (1996) *Virology* 219:161); a peptide nucleic acid (Hirshman et al. (1996) *J. Invest. Med.* 44:347); an antisense molecule (Bordier et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:9383) which can be used to down-regulate the expression or synthesis of aberrant or foreign proteins, such as HIV proteins or a wide variety of oncogenes such as p53 (Hesketh, *The Oncogene Facts Book*, Academic Press, New York, (1995); a biopharmaceutical agent or antisense molecule used to treat HIV-infection, such as an inhibitor of p24 (Nakashima et al. (1994) *Nucleic Acids Res.* 22:5004); or reverse-transcriptase (see, Bordier, *supra*).

Other proteins of therapeutic interest can be expressed *in vivo* by gene transfer vectors using the methods of the invention. For instance sustained *in vivo* expression of tissue factor inhibitory protein (TFPI) is useful for treatment of conditions including sepsis and DIC and in preventing reperfusion injury. (See International Publications Nos. WO 93/24143, WO 93/25230 and WO 96/06637). Nucleic acid sequences encoding various forms of TFPI can be obtained, for example, as described in US Patent Nos. 4,966,852; 5,106,833; and 5,466,783, and incorporated into the gene transfer vectors described herein.

Erythropoietin (EPO) and leptin can also be expressed *in vivo* from genetically modified T cells according to the methods of the invention. For instance EPO is useful in gene therapy treatment of a variety of disorders including anemia (see International Publication No. WO 95/13376 entitled "Gene Therapy for Treatment of Anemia"). Sustained delivery of leptin by the methods of the invention is useful in treatment of obesity. See International Publication No. WO 96/05309 for a description of the leptin gene and the use thereof in the treatment of obesity.

A variety of other disorders can also be treated by the methods of the invention. For example, sustained *in vivo* systemic production of apolipoprotein E or apolipoprotein A from genetically modified T cells can be used for treatment of hyperlipidemia (see Breslow et al. (1994) *Biotechnology* 12:365). Sustained production of angiotensin receptor inhibitor (Goodfriend et al. (1996) *N. Engl. J. Med.* 334:1469) can be provided by the methods described herein. As yet an additional example, the long term *in vivo* systemic production of angiostatin is useful in the treatment of a variety of tumors. (See O'Reilly et al. (1996) *Nature Med.* 2:689).

In other embodiments, gene transfer vectors can be constructed to encode a cytokine or other immunomodulatory molecule. For example, nucleic acid sequences encoding native IL-2 and gamma-interferon can be obtained as described in US Patent Nos. 4,738,927 and 5,326,859, respectively, while useful muteins of these proteins can be obtained as described in U.S. Patent No. 4,853,332. Nucleic acid sequences encoding the short and long forms of mCSF can be obtained as described in US Patent Nos. 4,847,201 and 4,879,227, respectively. In particular aspects of the invention, retroviral vectors

expressing cytokine or immunomodulatory genes can be produced as described herein (for example, employing the packaging cell lines of the present invention) and in International Application No. PCT US 94/02951, entitled
5 "Compositions and Methods for Cancer Immunotherapy."

Examples of suitable immunomodulatory molecules for use herein include the following: IL-1 and IL-2 (Karupiah et al. (1990) *J. Immunology* 144:290-298, Weber et al. (1987) *J. Exp. Med.* 166:1716-1733, Gansbacher et al. (1990) *J. Exp. Med.* 172:1217-1224, and U.S. Patent No. 4,738,927); IL-3 and IL-4 (Tepper et al. (1989) *Cell* 57:503-512, Golumbek et al. (1991) *Science* 254:713-716, and U.S. Patent No. 5,017,691); IL-5 and IL-6 (Brakenhof et al. (1987) *J. Immunol.* 139:4116-4121, and
15 International Publication No. WO 90/06370); IL-7 (U.S. Patent No. 4,965,195); IL-8, IL-9, IL-10, IL-11, IL-12, and IL-13 (*Cytokine Bulletin*, Summer 1994); IL-14 and IL-15; alpha interferon (Finter et al. (1991) *Drugs* 42:749-765, U.S. Patent Nos. 4,892,743 and 4,966,843, International Publication No. WO 85/02862, Nagata et al. (1980) *Nature* 284:316-320, Familletti et al. (1981) *Methods in Enz.* 78:387-394, Twu et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2046-2050, and Faktor et al. (1990) *Oncogene* 5:867-872); beta-interferon (Seif et al. (1991) *J. Virol.* 65:664-671); gamma-interferons (Radford et al. (1991) *The American Society of Hepatology* 20082015, Watanabe et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:9456-9460, Gansbacher et al. (1990) *Cancer Research* 50:7820-7825, Maio et al. (1989) *Can. Immunol. Immunother.* 30:34-42, and U.S. Patent Nos. 4,762,791 and 4,727,138); G-CSF (U.S. Patent Nos. 4,999,291 and 4,810,643); GM-CSF (International Publication No. WO 85/04188); tumor necrosis factors (TNFs) (Jayaraman et al. (1990) *J. Immunology* 144:942-951); CD3 (Krissanen et

al. (1987) *Immunogenetics* 26:258-266); ICAM-1 (Altman et al. (1989) *Nature* 338:512-514, Simmons et al. (1988) *Nature* 331:624-627); ICAM-2, LFA-1, LFA-3 (Wallner et al. (1987) *J. Exp. Med.* 166:923-932); MHC class I molecules, 5 MHC class II molecules, B7.1-.3, β_2 -microglobulin (Parnes et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:2253-2257); chaperones such as calnexin; and MHC-linked transporter proteins or analogs thereof (Powis et al. (1991) *Nature* 354:528-531). Immunomodulatory factors may also be 10 agonists, antagonists, or ligands for these molecules. For example, soluble forms of receptors can often behave as antagonists for these types of factors, as can mutated forms of the factors themselves.

Nucleic acid molecules that encode the above- 15 described substances, as well as other nucleic acid molecules that are advantageous for use within the present invention, may be readily obtained from a variety of sources, including, for example, depositories such as the American Type Culture Collection, or from commercial 20 sources such as British Bio-Technology Limited (Cowley, Oxford England). Representative examples include BBG 12 (containing the GM-CSF gene coding for the mature protein of 127 amino acids), BBG 6 (which contains sequences encoding gamma interferon), A.T.C.C. Deposit No. 39656 25 (which contains sequences encoding TNF), A.T.C.C. Deposit No. 20663 (which contains sequences encoding alpha-interferon), A.T.C.C. Deposit Nos. 31902, 31902 and 39517 (which contain sequences encoding beta-interferon), A.T.C.C. Deposit No. 67024 (which contains a sequence 30 which encodes Interleukin-1b), A.T.C.C. Deposit Nos. 39405, 39452, 39516, 39626 and 39673 (which contain sequences encoding Interleukin-2), A.T.C.C. Deposit Nos. 59399, 59398, and 67326 (which contain sequences encoding Interleukin-3), A.T.C.C. Deposit No. 57592 (which

contains sequences encoding Interleukin-4), A.T.C.C. Deposit Nos. 59394 and 59395 (which contain sequences encoding Interleukin-5), and A.T.C.C. Deposit No. 67153 (which contains sequences encoding Interleukin-6).

5 Plasmids containing cytokine genes or immunomodulatory genes (International Publication Nos. WO 94/02951 and WO 96/21015) can be digested with appropriate restriction enzymes, and DNA fragments containing the particular gene of interest can be inserted into a gene transfer vector using standard molecular biology techniques. (See, e.g., Sambrook et al., *supra.*, or Ausubel et al. (eds) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience).

10 Exemplary hormones, growth factors and other proteins which are useful for long term expression are described, for example, in European Publication No. 0437478B1, entitled "Cyclodextrin-Peptide Complexes." Nucleic acid sequences encoding a variety of hormones can be used, including those encoding human growth hormone, 15 insulin, calcitonin, prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (HCG), and thyroid stimulating hormone (TSH). A variety of different forms of IGF-1 and IGF-2 growth factor polypeptides are also well known the art and can be incorporated into gene transfer vectors for long term expression *in vivo*. See, e.g., European Patent 20 No. 0123228B1, published for grant September 19, 1993, entitled "Hybrid DNA Synthesis of Mature Insulin-like Growth Factors." As an additional example, the long term *in vivo* expression of different forms of fibroblast growth factor can also be effected employing the compositions and methods of invention. See, e.g., U.S. Patent Nos. 5,464,774, 5,155,214, and 4,994,559 for a description of different fibroblast growth factors.

Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene
5 from a vector known to include the same. For example, plasmids which contain sequences that encode altered cellular products may be obtained from a depository such as the A.T.C.C., or from commercial sources. Plasmids containing the nucleotide sequences of interest can be
10 digested with appropriate restriction enzymes, and DNA fragments containing the nucleotide sequences can be inserted into a gene transfer vector using standard molecular biology techniques.

Alternatively, cDNA sequences for use with the
15 present invention may be obtained from cells which express or contain the sequences, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA.
20 Briefly, mRNA from a cell which expresses the gene of interest can be reverse transcribed with reverse transcriptase using oligo-dT or random primers. The single stranded cDNA may then be amplified by PCR (see U.S. Patent Nos. 4,683,202, 4,683,195 and 4,800,159, see
25 also *PCR Technology: Principles and Applications for DNA Amplification*, Erlich (ed.), Stockton Press, 1989)) using oligonucleotide primers complementary to sequences on either side of desired sequences.

The nucleotide sequence of interest can also be
30 produced synthetically, rather than cloned, using a DNA synthesizer (e.g., an Applied Biosystems Model 392 DNA Synthesizer, available from ABI, Foster City, California). The nucleotide sequence can be designed with the appropriate codons for the expression product

desired. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) *Nature* 292:756; Nambair et al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol. Chem.* 259:6311.

The synthetic expression cassettes of the present invention can be employed in the construction of packaging cell lines for use with retroviral vectors.

One type of retrovirus, the murine leukemia virus, or "MLV", has been widely utilized for gene therapy applications (see generally Mann et al. (*Cell* 33:153, 1993), Cane and Mulligan (*Proc, Nat'l. Acad. Sci. USA* 81:6349, 1984), and Miller et al., *Human Gene Therapy* 1:5-14, 1990).

Lentiviral vectors typically, comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to one or more genes of interest, an origin of second strand DNA synthesis and a 3' lentiviral LTR, wherein the lentiviral vector contains a nuclear transport element. The nuclear transport element may be located either upstream (5') or downstream (3') of a coding sequence of interest. Within certain embodiments, the nuclear transport element is not RRE. Within one embodiment the packaging signal is an extended packaging signal. Within other embodiments the promoter is a tissue specific promoter, or, alternatively, a promoter such as CMV. Within other embodiments, the lentiviral vector further comprises an internal ribosome entry site.

A wide variety of lentiviruses may be utilized within the context of the present invention, including for example, lentiviruses selected from the group consisting of HIV, HIV-1, HIV-2, FIV and SIV.

In one embodiment of the present invention synthetic Env and/or Gag-polymerase expression cassettes are provided comprising a promoter and a sequence encoding synthetic Gag-polymerase (SEQ ID NO:6) and at least one
5 of vpr, vpu, nef or vif, wherein the promoter is operably linked to Gag-polymerase and vpr, vpu, nef or vif.

Within yet another aspect of the invention, host cells (e.g., packaging cell lines) are provided which contain any of the expression cassettes described herein.
10 For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence encoding synthetic Env and/or Gag-polymerase, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding Env and/or Gag-
15 polymerase. Packaging cell lines may further comprise a promoter and a sequence encoding tat, rev, or an envelope, wherein the promoter is operably linked to the sequence encoding tat, rev, or, the envelope. The packaging cell line may further comprise a sequence
20 encoding any one or more of nef, vif, vpu or vpr.

In one embodiment, the expression cassette (carrying, for example, the synthetic Env, synthetic tat and/or synthetic Gag-polymerase) is stably integrated. The packaging cell line, upon introduction of a
25 lentiviral vector, typically produces viral particles. The promoter regulating expression of the synthetic expression cassette may be inducible. Typically, the packaging cell line, upon introduction of a lentiviral vector, produces viral particles that are essentially
30 free of replication competent virus.

Packaging cell lines are provided comprising an expression cassette which directs the expression of a synthetic Env (or Gag-polymerase) gene, an expression cassette which directs the expression of a Gag (or Env)

gene optimized for expression (e.g., Andre, S., et al., *Journal of Virology* 72(2):1497-1503, 1998; Haas, J., et al., *Current Biology* 6(3):315-324, 1996). A lentiviral vector is introduced into the packaging cell line to
5 produce a vector particle producing cell line.

As noted above, lentiviral vectors can be designed to carry or express a selected gene(s) or sequences of interest. Lentiviral vectors may be readily constructed from a wide variety of lentiviruses (see RNA Tumor
10 Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Representative examples of lentiviruses included HIV, HIV-1, HIV-2, FIV and SIV. Such lentiviruses may either be obtained from patient isolates, or, more preferably, from depositories or collections such as the
15 American Type Culture Collection, or isolated from known sources using available techniques.

Portions of the lentiviral gene delivery vectors (or vehicles) may be derived from different viruses. For example, in a given recombinant lentiviral vector, LTRs
20 may be derived from an HIV, a packaging signal from SIV, and an origin of second strand synthesis from HrV-2. Lentiviral vector constructs may comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, one or more heterologous sequences, an origin of second strand DNA
25 synthesis and a 3' LTR, wherein said lentiviral vector contains a nuclear transport element that is not RRE.

Briefly, Long Terminal Repeats ("LTRs") are subdivided into three elements, designated U5, R and U3. These elements contain a variety of signals which are
30 responsible for the biological activity of a retrovirus, including for example, promoter and enhancer elements which are located within U3. LTRs may be readily identified in the provirus (integrated DNA form) due to their precise duplication at either end of the genome.

As utilized herein, a 5' LTR should be understood to include a 5' promoter element and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector. The 3' LTR should be understood to include a polyadenylation signal, and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector.

The tRNA binding site and origin of second strand DNA synthesis are also important for a retrovirus to be biologically active, and may be readily identified by one of skill in the art. For example, retroviral tRNA binds to a tRNA binding site by Watson-Crick base pairing, and is carried with the retrovirus genome into a viral particle. The tRNA is then utilized as a primer for DNA synthesis by reverse transcriptase. The tRNA binding site may be readily identified based upon its location just downstream from the 5'LTR. Similarly, the origin of second strand DNA synthesis is, as its name implies, important for the second strand DNA synthesis of a retrovirus. This region, which is also referred to as the poly-purine tract, is located just upstream of the 3'LTR.

In addition to a 5' and 3' LTR, tRNA binding site, and origin of second strand DNA synthesis, recombinant retroviral vector constructs may also comprise a packaging signal, as well as one or more genes or coding sequences of interest. In addition, the lentiviral vectors have a nuclear transport element which, in preferred embodiments is not RRE. Representative examples of suitable nuclear transport elements include the element in Rous sarcoma virus (Ogert, et al., J ViroL 70, 3834-3843, 1996), the element in Rous sarcoma virus (Liu & Mertz, Genes & Dev., 9, 1766-1789, 1995) and the element in the genome of simian retrovirus type I

(Zolotukhin, et al., *J Virol.* 68, 7944-7952, 1994).

Other potential elements include the elements in the histone gene (Kedes, *Annu. Rev. Biochem.* 48, 837-870, 1970), the α -interferon gene (Nagata et al., *Nature* 287, 401-408, 1980), the β -adrenergic receptor gene (Koilkka, et al., *Nature* 329, 75-79, 1987), and the c-Jun gene (Hattorie, et al., *Proc. Natl. Acad. Sci. USA* 85, 9148-9152, 1988).

Recombinant lentiviral vector constructs typically lack both *Gag*-polymerase and *env* coding sequences. Recombinant lentiviral vector typically contain less than 20, preferably 15, more preferably 10, and most preferably 8 consecutive nucleotides found in *Gag*-polymerase or *env* genes. One advantage of the present invention is that the synthetic *Gag*-polymerase expression cassettes, which can be used to construct packaging cell lines for the recombinant retroviral vector constructs, have little homology to wild-type *Gag*-polymerase sequences and thus considerably reduce or eliminate the possibility of homologous recombination between the synthetic and wild-type sequences.

Lentiviral vectors may also include tissue-specific promoters to drive expression of one or more genes or sequences of interest. For example, lentiviral vector particles of the invention can contain a liver specific promoter to maximize the potential for liver specific expression of the exogenous DNA sequence contained in the vectors. Preferred liver specific promoters include the hepatitis B X-gene promoter and the hepatitis B core protein promoter. These liver specific promoters are preferably employed with their respective enhancers. The enhancer element can be linked at either the 5' or the 3' end of the nucleic acid encoding the sequences of interest. The hepatitis B X gene promoter and its

enhancer can be obtained from the viral genome as a 332 base pair *EcoRV-NcoI* DNA fragment employing the methods described in Twu, et al., *J Virol.* 61:3448-3453, 1987. The hepatitis B core protein promoter can be obtained
5 from the viral genome as a 584 base pair *BamHI-BglIII* DNA fragment employing the methods described in Gerlach, et al., *Virol* 189:59-66, 1992. It may be necessary to remove the negative regulatory sequence in the *BamHI-BglIII* fragment prior to inserting it. Other liver
10 specific promoters include the AFP (alpha fetal protein) gene promoter and the albumin gene promoter, as disclosed in EP Patent Publication 0 415 731, the -1 antitrypsin gene promoter, as disclosed in Rettenger, et al., *Proc. Natl. Acad. Sci.* 91:1460-1464, 1994, the fibrinogen
15 gene promoter, the APO-A1 (Apolipoprotein A1) gene promoter, and the promoter genes for liver transference enzymes such as, for example, SGOT, SGPT and glutamyle transferase. See also PCT Patent Publications WO 90/07936 and WO 91/02805 for a description of the use of
20 liver specific promoters in lentiviral vector particles.

Lentiviral vector constructs may be generated such that more than one gene of interest is expressed. This may be accomplished through the use of di- or oligo-cistronic cassettes (e.g., where the coding regions are
25 separated by 80 nucleotides or less, see generally Levin et al., *Gene* 108:167-174, 1991), or through the use of Internal Ribosome Entry Sites ("IRES").

Packaging cell lines suitable for use with the above described recombinant retroviral vector constructs may be
30 readily prepared given the disclosure provided herein. Briefly, the parent cell line from which the packaging cell line is derived can be selected from a variety of

mammalian cell lines, including for example, 293, RD, COS-7, CHO, BHK, VERO, HT1080, and myeloma cells.

After selection of a suitable host cell for the generation of a packaging cell line, one or more
5 expression cassettes are introduced into the cell line in order to complement or supply in trans components of the vector which have been deleted.

Representative examples of suitable expression cassettes have been described herein and include
10 synthetic Env, tat, Gag, synthetic Gag-protease, synthetic Gag-reverse transcriptase and synthetic Gag-polymerase expression cassettes, which comprise a promoter and a sequence encoding, e.g., Env, tat, or Gag-polymerase and at least one of vpr, vpu, nef or vif,
15 wherein the promoter is operably linked to Env, tat or Gag-polymerase and vpr, vpu, nef or vif. As described above, optimized Env, Gag and/or tat coding sequences may also be utilized in various combinations in the generation of packaging cell lines.

20 Utilizing the above-described expression cassettes, a wide variety of packaging cell lines can be generated. For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence encoding synthetic HIV (e.g., Gag, Env, tat,
25 Gag-polymerase, Gag-reverse transcriptase or Gag-protease) polypeptide, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding the HIV polypeptide. Within other aspects, packaging cell lines are provided comprising a promoter
30 and a sequence encoding Gag, tat, rev, or an envelope (e.g., HIV env), wherein the promoter is operably linked to the sequence encoding Gag, tat, rev, or, the envelope. Within further embodiments, the packaging cell line may comprise a sequence encoding any one or more of nef, vif,

vpu or vpr. For example, the packaging cell line may contain only nef, vif, vpu, or vpr alone, nef and vif, nef and vpu, nef and vpr, vif and vpu, vif and vpr, vpu and vpr, nef vif and vpu, nef vif and vpr, nef vpu and vpr, vvir vpu and vpr, or, all four of nef vif vpu and vpr.

In one embodiment, the expression cassette is stably integrated. Within another embodiment, the packaging cell line, upon introduction of a lentiviral vector, produces particles. Within further embodiments the promoter is inducible. Within certain preferred embodiments of the invention, the packaging cell line, upon introduction of a lentiviral vector, produces particles that are free of replication competent virus.

The synthetic cassettes containing optimized coding sequences are transfected into a selected cell line. Transfected cells are selected that (i) carry, typically, integrated, stable copies of the Gag, Pol, and Env coding sequences, and (ii) are expressing acceptable levels of these polypeptides (expression can be evaluated by methods known in the prior art, e.g., see Examples 1-4). The ability of the cell line to produce VLPs may also be verified (Examples 6, 7 and 15).

A sequence of interest is constructed into a suitable viral vector as discussed above. This defective virus is then transfected into the packaging cell line. The packaging cell line provides the viral functions necessary for producing virus-like particles into which the defective viral genome, containing the sequence of interest, are packaged. These VLPs are then isolated and can be used, for example, in gene delivery or gene therapy.

Further, such packaging cell lines can also be used to produce VLPs alone, which can, for example, be used as

adjuvants for administration with other antigens or in vaccine compositions. Also, co-expression of a selected sequence of interest encoding a polypeptide (for example, an antigen) in the packaging cell line can also result in the entrapment and/or association of the selected polypeptide in/with the VLPs.

2.3 DNA IMMUNIZATION AND GENE DELIVERY

A variety of polypeptide antigens can be used in the practice of the present invention. Polypeptide antigens can be included in DNA immunization constructs containing, for example, any of the synthetic expression cassettes described herein fused in-frame to a coding sequence for the polypeptide antigen, where expression of the construct results in VLPs presenting the antigen of interest. Antigens can be derived from a wide variety of viruses, bacteria, fungi, plants, protozoans and other parasites. For example, the present invention will find use for stimulating an immune response against a wide variety of proteins from the herpesvirus family, including proteins derived from herpes simplex virus (HSV) types 1 and 2, such as HSV-1 and HSV-2 gB, gD, gH, VP16 and VP22; antigens derived from varicella zoster virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) including CMV gB and gH; and antigens derived from other human herpesviruses such as HHV6 and HHV7. (See, e.g. Chee et al., *Cytomegaloviruses* (J.K. McDougall, ed., Springer-Verlag 1990) pp. 125-169, for a review of the protein coding content of cytomegalovirus; McGeoch et al., *J. Gen. Virol.* (1988) 69:1531-1574, for a discussion of the various HSV-1 encoded proteins; U.S. Patent No. 5,171,568 for a discussion of HSV-1 and HSV-2 gB and gD proteins and the genes encoding therefore; Baer et al., *Nature* (1984) 310:207-211, for the identification of

protein coding sequences in an EBV genome; and Davison and Scott, *J. Gen. Virol.* (1986) 67:1759-1816, for a review of VZV.)

5 Additionally, immune responses to antigens from the hepatitis family of viruses, including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the delta hepatitis virus (HDV), hepatitis E virus (HEV), and hepatitis G virus, can also be stimulated using the constructs of the present invention. By way of example,
10 the HCV genome encodes several viral proteins, including E1 (also known as E) and E2 (also known as E2/NSI), which will find use with the present invention (see, Houghton et al., *Hepatology* (1991) 14:381-388, for a discussion of HCV proteins, including E1 and E2). The δ -antigen from
15 HDV can also be used (see, e.g., U.S. Patent No. 5,389,528, for a description of the δ -antigen).

Similarly, influenza virus is another example of a virus for which the present invention will be particularly useful. Specifically, the envelope
20 glycoproteins HA and NA of influenza A are of particular interest for generating an immune response. Numerous HA subtypes of influenza A have been identified (Kawaoka et al., *Virology* (1990) 179:759-767; Webster et al. "Antigenic variation among type A influenza viruses," p.
25 127-168. In: P. Palese and D.W. Kingsbury (ed.), *Genetics of influenza viruses*. Springer-Verlag, New York).

Other antigens of particular interest to be used in the practice of the present invention include antigens and polypeptides derived therefrom from human
30 papillomavirus (HPV), such as one or more of the various early proteins including E6 and E7; tick-borne encephalitis viruses; and HIV-1 (also known as HTLV-III, LAV, ARV, etc.), including, but not limited to, antigens such as gp120, gp41, gp160, Gag and pol from a variety of

isolates including, but not limited to, HIV_{IIIB}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}). See, e.g., Myers, et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico; Myers, et al., *Human Retroviruses and Aids*, 1990, Los Alamos, New Mexico: Los Alamos National Laboratory.

10 Proteins derived from other viruses will also find use in the claimed methods, such as without limitation, proteins from members of the families Picornaviridae (e.g., polioviruses, etc.); Caliciviridae; Togaviridae (e.g., rubella virus, dengue virus, etc.); Flaviviridae; 15 Coronaviridae; Reoviridae; Birnaviridae; Rhabdoviridae (e.g., rabies virus, etc.); Filoviridae; Paramyxoviridae (e.g., mumps virus, measles virus, respiratory syncytial virus, etc.); Orthomyxoviridae (e.g., influenza virus types A, B and C, etc.); Bunyaviridae; Arenaviridae; 20 Retroviridae, e.g., HTLV-I; HTLV-II; HIV-1; HIV-2; simian immunodeficiency virus (SIV) among others. See, e.g. Virology, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991; Virology, 3rd Edition (Fields, BN, DM Knipe, PM 25 Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA) for a description of these and other viruses.

Particularly preferred bacterial antigens are derived from organisms that cause diphtheria, tetanus, pertussis, meningitis, and other pathogenic states, 30 including, without limitation, antigens derived from *Corynebacterium diphtheriae*, *Clostridium tetani*, *Bordetella pertussis*, *Neisseria meningitidis*, including serotypes Meningococcus A, B, C, Y and WI35 (MenA, B, C, Y and WI35), *Haemophilus influenza* type B (Hib), and

Helicobacter pylori. Examples of parasitic antigens include those derived from organisms causing malaria, tuberculosis, and Lyme disease.

Furthermore, the methods described herein provide means for treating a variety of malignant cancers. For example, the system of the present invention can be used to enhance both humoral and cell-mediated immune responses to particular proteins specific to a cancer in question, such as an activated oncogene, a fetal antigen, or an activation marker. Such tumor antigens include any of the various MAGEs (melanoma associated antigen E), including MAGE 1, 2, 3, 4, etc. (Boon, T. Scientific American (March 1993):82-89); any of the various tyrosinases; MART 1 (melanoma antigen recognized by T cells), mutant ras; mutant p53; p97 melanoma antigen; CEA (carcinoembryonic antigen), among others.

DNA immunization using synthetic expression cassettes of the present invention has been demonstrated to be efficacious (Examples 8 and 10-12). Animals were immunized with both the synthetic expression cassette and the wild type expression cassette. The results of the immunizations with plasmid-DNAs showed that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression cassettes. Also, the second boost immunization induced a secondary immune response, for example after two to eight weeks. Further, the results of CTL assays showed increased potency of synthetic expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

It is readily apparent that the subject invention can be used to mount an immune response to a wide variety of antigens and hence to treat or prevent a large number of diseases.

2.3.1 DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF THE
PRESENT INVENTION

Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant
5 methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. The sequences can be analyzed by conventional sequencing techniques. Furthermore, the desired gene can be isolated directly
10 from cells and tissues containing the same, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain, isolate and sequence DNA. Once the sequence is known, the gene
15 of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will
20 be expressed. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al., *Science* (1984) 223:1299; Jay et al., *J. Biol. Chem.* (1984) 259:6311; Stemmer, W.P.C., (1995) *Gene* 164:49-53.

Next, the gene sequence encoding the desired antigen can be inserted into a vector containing a synthetic
expression cassette of the present invention (e.g., see
30 Example 1 for construction of various exemplary synthetic expression cassette). The antigen is inserted into the synthetic coding sequence such that when the combined sequence is expressed it results in the production of VLPs comprising the polypeptide and/or the antigen of

interest. Insertions can be made within the Gag coding sequence or at either end of the coding sequence (5', amino terminus of the expressed polypeptide; or 3', carboxy terminus of the expressed polypeptide -- e.g., see Example 1) (Wagner, R., et al., *Arch Virol.* 127:117-137, 1992; Wagner, R., et al., *Virology* 200:162-175, 1994; Wu, X., et al., *J. Virol.* 69(6):3389-3398, 1995; Wang, C-T., et al., *Virology* 200:524-534, 1994; Chazal, N., et al., *Virology* 68(1):111-122, 1994; Griffiths, J.C., et al., *J. Virol.* 67(6):3191-3198, 1993; Reicin, A.S., et al., *J. Virol.* 69(2):642-650, 1995).

Up to 50% of the coding sequences of p55Gag can be deleted without affecting the assembly to virus-like particles and expression efficiency (Borsetti, A., et al., *J. Virol.* 72(11):9313-9317, 1998; Gamier, L., et al., *J. Virol.* 72(6):4667-4677, 1998; Zhang, Y., et al., *J. Virol.* 72(3):1782-1789, 1998; Wang, C., et al., *J. Virol.* 72(10):7950-7959, 1998). In one embodiment of the present invention, immunogenicity of the high level expressing synthetic p55GagMod and p55GagProtMod expression cassettes can be increased by the insertion of different structural or non-structural HIV antigens, multiepitope cassettes, or cytokine sequences into deleted, mutated or truncated regions of p55GagMod sequence. In another embodiment of the present invention, immunogenicity of the high level expressing synthetic Env expression cassettes can be increased by the insertion of different structural or non-structural HIV antigens, multiepitope cassettes, or cytokine sequences into deleted regions of gp120Mod, gp140Mod or gp160Mod sequences. Such deletions may be generated following the teachings of the present invention and information available to one of ordinary skill in the art. One possible advantage of this approach, relative to using full-length modified Env

sequences fused to heterologous polypeptides, can be higher expression/secretion efficiency and/or higher immunogenicity of the expression product. Such deletions may be generated following the teachings of the present invention and information available to one of ordinary skill in the art. One possible advantage of this approach, relative to using full-length Env, Gag or Tat sequences fused to heterologous polypeptides, can be higher expression/secretion efficiency and/or immunogenicity of the expression product.

When sequences are added to the amino terminal end of Gag (for example, when using the synthetic p55GagMod expression cassette of the present invention), the polynucleotide can contain coding sequences at the 5' end that encode a signal for addition of a myristic moiety to the Gag-containing polypeptide (e.g., sequences that encode Met-Gly).

The ability of Gag-containing polypeptide constructs to form VLPs can be empirically determined following the teachings of the present specification.

HIV polypeptide/antigen synthetic expression cassettes include control elements operably linked to the coding sequence, which allow for the expression of the gene *in vivo* in the subject species. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter, the mouse mammary tumor virus LTR promoter, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop

codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived
5 from SV40, as described in Sambrook et al., *supra*, as well as a bovine growth hormone terminator sequence.

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as
10 described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart
15 et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence.

Furthermore, plasmids can be constructed which include a chimeric antigen-coding gene sequences, encoding, e.g., multiple antigens/epitopes of interest,
20 for example derived from a single or from more than one viral isolate.

Typically the antigen coding sequences precede or follow the synthetic coding sequences and the chimeric transcription unit will have a single open reading frame
25 encoding both the antigen of interest and the synthetic Gag coding sequences. Alternatively, multi-cistronic cassettes (e.g., bi-cistronic cassettes) can be constructed allowing expression of multiple antigens from a single mRNA using the EMCV IRES, or the like. Lastly,
30 antigens can be encoded on separate transcripts from independent promoters on a single plasmid or other vector.

Once complete, the constructs are used for nucleic acid immunization or the like using standard gene

delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. Genes can be delivered either directly to the vertebrate subject or, alternatively, delivered ex vivo, to cells derived from the subject and the cells reimplanted in the subject.

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. Selected sequences can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems have been described (U.S. Patent No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy* (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994) 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery.

AAV vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769 (published 4 March 1993); Lebkowski et al., *Molec. Cell. Biol.* (1988) 8:3988-3996; Vincent et al., *Vaccines* 90 (1990) (Cold Spring Harbor Laboratory Press); Carter, B.J. *Current Opinion in Biotechnology* (1992) 3:533-539; Muzyczka, N. *Current Topics in Microbiol. and Immunol.* (1992) 158:97-129; Kotin, R.M. *Human Gene Therapy* (1994) 5:793-801; Shelling and Smith, *Gene Therapy* (1994) 1:165-169; and Zhou et al., *J. Exp. Med.* (1994) 179:1867-1875.

Another vector system useful for delivering the polynucleotides of the present invention is the enterically administered recombinant poxvirus vaccines described by Small, Jr., P.A., et al. (U.S. Patent No. 5,676,950, issued October 14, 1997).

Additional viral vectors which will find use for delivering the nucleic acid molecules encoding the antigens of interest include those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the genes can be constructed as follows. The DNA encoding the particular synthetic Gag/antigen coding sequence is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the coding sequences of interest into the viral genome. The resulting TK recombinant can be selected by culturing the

cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the genes. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g., WO 91/12882; WO 89/03429; and WO 92/03545.

Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al., *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al., *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery.

Members of the Alphavirus genus, such as, but not limited to, vectors derived from the Sindbis, Semliki Forest, and Venezuelan Equine Encephalitis viruses, will also find use as viral vectors for delivering the polynucleotides of the present invention (for example, a synthetic Gag- or Env-polypeptide encoding expression cassette as described in Example 14 below). For a description of Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., *J. Virol.* (1996) 70:508-519; and International Publication Nos. WO 95/07995 and WO 96/17072; as well as, Dubensky, Jr., T.W., et al., U.S. Patent No. 5,843,723,

issued December 1, 1998, and Dubensky, Jr., T.W., U.S. Patent No. 5,789,245, issued August 4, 1998.

5 A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient expression of the coding sequences of interest (for example, a synthetic Gag/HCV-core expression cassette) in a host cell. In this system, cells are first infected in vitro with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase
10 displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus
15 recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products. See,
20 e.g., Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* (1990) 87:6743-6747; Fuerst et al., *Proc. Natl. Acad. Sci. USA* (1986) 83:8122-8126.

As an alternative approach to infection with vaccinia or avipox virus recombinants, or to the delivery
25 of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region for T7 RNA polymerase can be engineered.
30 Translation of RNA derived from this template will generate T7 RNA polymerase which in turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from

translation of the amplification template RNA will lead to transcription of the desired gene. Because some T7 RNA polymerase is required to initiate the amplification, T7 RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, e.g., International Publication No. WO 94/26911; Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130; Deng and Wolff, *Gene* (1994) 143:245-249; Gao et al., *Biochem. Biophys. Res. Commun.* (1994) 200:1201-1206; Gao and Huang, *Nuc. Acids Res.* (1993) 21:2867-2872; Chen et al., *Nuc. Acids Res.* (1994) 22:2114-2120; and U.S. Patent No. 5,135,855.

The synthetic expression cassette of interest can also be delivered without a viral vector. For example, the synthetic expression cassette can be packaged as DNA or RNA in liposomes prior to delivery to the subject or to cells derived therefrom. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed DNA to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight, *Biochim. Biophys. Acta.* (1991) 1097:1-17; Straubinger et al., in *Methods of Enzymology* (1983), Vol. 101, pp. 512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations, with cationic liposomes particularly preferred. Cationic liposomes have been shown to mediate intracellular

delivery of plasmid DNA (Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416); mRNA (Malone et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081); and purified transcription factors (Debs et al., *J. Biol. Chem.* (1990) 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other commercially available lipids include (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; PCT Publication No. WO 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as, from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See, e.g., Straubinger et al., in *METHODS OF*

IMMUNOLOGY (1983), Vol. 101, pp. 512-527; Szoka et al.,
Proc. Natl. Acad. Sci. USA (1978) 75:4194-4198;
Papahadjopoulos et al., Biochim. Biophys. Acta (1975)
394:483; Wilson et al., Cell (1979) 17:77); Deamer and
5 Bangham, Biochim. Biophys. Acta (1976) 443:629; Ostro et
al., Biochem. Biophys. Res. Commun. (1977) 76:836; Fraley
et al., Proc. Natl. Acad. Sci. USA (1979) 76:3348); Enoch
and Strittmatter, Proc. Natl. Acad. Sci. USA (1979)
76:145); Fraley et al., J. Biol. Chem. (1980) 255:10431;
10 Szoka and Papahadjopoulos, Proc. Natl. Acad. Sci. USA
(1978) 75:145; and Schaefer-Ridder et al., Science (1982)
215:166.

The DNA and/or protein antigen(s) can also be
delivered in cochleate lipid compositions similar to
15 those described by Papahadjopoulos et al., Biochem.
Biophys. Acta. (1975) 394:483-491. See, also, U.S.
Patent Nos. 4,663,161 and 4,871,488.

The synthetic expression cassette of interest (e.g.,
any of the synthetic expression cassettes described in
20 Example 1) may also be encapsulated, adsorbed to, or
associated with, particulate carriers. Such carriers
present multiple copies of a selected antigen to the
immune system and promote migration, trapping and
retention of antigens in local lymph nodes. The
25 particles can be taken up by professional antigen
presenting cells such as macrophages and dendritic cells,
and/or can enhance antigen presentation through other
mechanisms such as stimulation of cytokine release.
Examples of particulate carriers include those derived
30 from polymethyl methacrylate polymers, as well as
microparticles derived from poly(lactides) and
poly(lactide-co-glycolides), known as PLG. See, e.g.,
Jeffery et al., Pharm. Res. (1993) 10:362-368; McGee JP,

et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993.

Furthermore, other particulate systems and polymers can be used for the *in vivo* or *ex vivo* delivery of the gene of interest. For example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules, are useful for transferring a nucleic acid of interest. Similarly, DEAE dextran-mediated transfection, calcium phosphate precipitation or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates including bentonite and kaolin, chromic oxide, magnesium silicate, talc, and the like, will find use with the present methods. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Peptoids (Zuckerman, R.N., et al., U.S. Patent No. 5,831,005, issued November 3, 1998) may also be used for delivery of a construct of the present invention.

Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are especially useful for delivering synthetic expression cassettes of the present invention. The particles are coated with the synthetic expression cassette(s) to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefore, see, e.g., U.S. Patent Nos. 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744. Also, needle-less injection systems can be used (Davis, H.L., et al, *Vaccine* 12:1503-1509, 1994; Bioject, Inc., Portland, OR).

Recombinant vectors carrying a synthetic expression cassette of the present invention are formulated into compositions for delivery to the vertebrate subject. These compositions may either be prophylactic (to prevent infection) or therapeutic (to treat disease after infection). The compositions will comprise a "therapeutically effective amount" of the gene of interest such that an amount of the antigen can be produced *in vivo* so that an immune response is generated in the individual to which it is administered. The exact amount necessary will vary depending on the subject being treated; the age and general condition of the subject to be treated; the capacity of the subject's immune system to synthesize antibodies; the degree of protection desired; the severity of the condition being treated; the particular antigen selected and its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of skill in the art. Thus, a "therapeutically effective amount" will fall in a relatively broad range that can be determined through routine trials.

The compositions will generally include one or more "pharmaceutically acceptable excipients or vehicles" such as water, saline, glycerol, polyethyleneglycol, hyaluronic acid, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, surfactants and the like, may be present in such vehicles. Certain facilitators of immunogenicity or of nucleic acid uptake and/or expression can also be included in the compositions or coadministered, such as, but not limited to, bupivacaine, cardiotoxin and sucrose.

Once formulated, the compositions of the invention can be administered directly to the subject (e.g., as

described above) or, alternatively, delivered ex vivo, to cells derived from the subject, using methods such as those described above. For example, methods for the ex vivo delivery and reimplantation of transformed cells into a subject are known in the art and can include, e.g., dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, lipofectamine and LT-1 mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) (with or without the corresponding antigen) in liposomes, and direct microinjection of the DNA into nuclei.

Direct delivery of synthetic expression cassette compositions in vivo will generally be accomplished with or without viral vectors, as described above, by injection using either a conventional syringe, needleless devices such as Bioject® or a gene gun, such as the Accell® gene delivery system (PowderJect Technologies, Inc., Oxford, England). The constructs can be delivered (e.g., injected) either subcutaneously, epidermally, intradermally, intramuscularly, intravenous, intramucosally (such as nasally, rectally and vaginally), intraperitoneally or orally. Delivery of DNA into cells of the epidermis is particularly preferred as this mode of administration provides access to skin-associated lymphoid cells and provides for a transient presence of DNA in the recipient. Other modes of administration include oral ingestion and pulmonary administration, suppositories, needle-less injection, transcutaneous and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule.

2.3.2 EX VIVO DELIVERY OF THE SYNTHETIC EXPRESSION
CASSETTES OF THE PRESENT INVENTION

In one embodiment, T cells, and related cell types (including but not limited to antigen presenting cells, such as, macrophage, monocytes, lymphoid cells, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof), can be used for ex vivo delivery of the synthetic expression cassettes of the present invention. T cells can be isolated from peripheral blood lymphocytes (PBLs) by a variety of procedures known to those skilled in the art. For example, T cell populations can be "enriched" from a population of PBLs through the removal of accessory and B cells. In particular, T cell enrichment can be accomplished by the elimination of non-T cells using anti-MHC class II monoclonal antibodies. Similarly, other antibodies can be used to deplete specific populations of non-T cells. For example, anti-Ig antibody molecules can be used to deplete B cells and anti-MacI antibody molecules can be used to deplete macrophages.

T cells can be further fractionated into a number of different subpopulations by techniques known to those skilled in the art. Two major subpopulations can be isolated based on their differential expression of the cell surface markers CD4 and CD8. For example, following the enrichment of T cells as described above, CD4⁺ cells can be enriched using antibodies specific for CD4 (see Coligan et al., supra). The antibodies may be coupled to a solid support such as magnetic beads. Conversely, CD8⁺ cells can be enriched through the use of antibodies specific for CD4 (to remove CD4⁺ cells), or can be isolated by the use of CD8 antibodies coupled to a solid support. CD4

lymphocytes from HIV-1 infected patients can be expanded ex vivo, before or after transduction as described by Wilson et. al. (1995) *J. Infect. Dis.* 172:88.

5 Following purification of T cells, a variety of methods of genetic modification known to those skilled in the art can be performed using non-viral or viral-based gene transfer vectors constructed as described herein. For example, one such approach involves transduction of
10 the purified T cell population with vector-containing supernatant of cultures derived from vector producing cells. A second approach involves co-cultivation of an irradiated monolayer of vector-producing cells with the purified T cells. A third approach involves a similar
15 co-cultivation approach; however, the purified T cells are pre-stimulated with various cytokines and cultured 48 hours prior to the co-cultivation with the irradiated vector producing cells. Pre-stimulation prior to such transduction increases effective gene transfer (Nolta et
20 al. (1992) *Exp. Hematol.* 20:1065). Stimulation of these cultures to proliferate also provides increased cell populations for re-infusion into the patient. Subsequent to co-cultivation, T cells are collected from the vector producing cell monolayer, expanded, and frozen in liquid
25 nitrogen.

Gene transfer vectors, containing one or more synthetic expression cassette of the present invention (associated with appropriate control elements for delivery to the isolated T cells) can be assembled using
30 known methods.

Selectable markers can also be used in the construction of gene transfer vectors. For example, a marker can be used which imparts to a mammalian cell transduced with the gene transfer vector resistance to a

cytotoxic agent. The cytotoxic agent can be, but is not limited to, neomycin, aminoglycoside, tetracycline, chloramphenicol, sulfonamide, actinomycin, netropsin, distamycin A, anthracycline, or pyrazinamide. For
5 example, neomycin phosphotransferase II imparts resistance to the neomycin analogue geneticin (G418).

The T cells can also be maintained in a medium containing at least one type of growth factor prior to being selected. A variety of growth factors are known in
10 the art which sustain the growth of a particular cell type. Examples of such growth factors are cytokine mitogens such as rIL-2, IL-10, IL-12, and IL-15, which promote growth and activation of lymphocytes. Certain types of cells are stimulated by other growth factors
15 such as hormones, including human chorionic gonadotropin (hCG) and human growth hormone. The selection of an appropriate growth factor for a particular cell population is readily accomplished by one of skill in the art.

20 For example, white blood cells such as differentiated progenitor and stem cells are stimulated by a variety of growth factors. More particularly, IL-3, IL-4, IL-5, IL-6, IL-9, GM-CSF, M-CSF, and G-CSF, produced by activated T_H and activated macrophages,
25 stimulate myeloid stem cells, which then differentiate into pluripotent stem cells, granulocyte-monocyte progenitors, eosinophil progenitors, basophil progenitors, megakaryocytes, and erythroid progenitors. Differentiation is modulated by growth factors such as
30 GM-CSF, IL-3, IL-6, IL-11, and EPO.

Pluripotent stem cells then differentiate into lymphoid stem cells, bone marrow stromal cells, T cell progenitors, B cell progenitors, thymocytes, T_H Cells, T_C cells, and B cells. This differentiation is modulated by

growth factors such as IL-3, IL-4, IL-6, IL-7, GM-CSF, M-CSF, G-CSF, IL-2, and IL-5.

Granulocyte-monocyte progenitors differentiate to monocytes, macrophages, and neutrophils. Such
5 differentiation is modulated by the growth factors GM-CSF, M-CSF, and IL-8. Eosinophil progenitors differentiate into eosinophils. This process is modulated by GM-CSF and IL-5.

The differentiation of basophil progenitors into
10 mast cells and basophils is modulated by GM-CSF, IL-4, and IL-9. Megakaryocytes produce platelets in response to GM-CSF, EPO, and IL-6. Erythroid progenitor cells differentiate into red blood cells in response to EPO.

Thus, during activation by the CD3-binding agent, T
15 cells can also be contacted with a mitogen, for example a cytokine such as IL-2. In particularly preferred embodiments, the IL-2 is added to the population of T cells at a concentration of about 50 to 100 $\mu\text{g/ml}$. Activation with the CD3-binding agent can be carried out
20 for 2 to 4 days.

Once suitably activated, the T cells are genetically modified by contacting the same with a suitable gene transfer vector under conditions that allow for transfection of the vectors into the T cells. Genetic
25 modification is carried out when the cell density of the T cell population is between about 0.1×10^6 and 5×10^6 , preferably between about 0.5×10^6 and 2×10^6 . A number of suitable viral and nonviral-based gene transfer vectors have been described for use herein.

30 After transduction, transduced cells are selected away from non-transduced cells using known techniques. For example, if the gene transfer vector used in the transduction includes a selectable marker which confers resistance to a cytotoxic agent, the cells can be

contacted with the appropriate cytotoxic agent, whereby non-transduced cells can be negatively selected away from the transduced cells. If the selectable marker is a cell surface marker, the cells can be contacted with a binding agent specific for the particular cell surface marker, whereby the transduced cells can be positively selected away from the population. The selection step can also entail fluorescence-activated cell sorting (FACS) techniques, such as where FACS is used to select cells from the population containing a particular surface marker, or the selection step can entail the use of magnetically responsive particles as retrievable supports for target cell capture and/or background removal.

More particularly, positive selection of the transduced cells can be performed using a FACS cell sorter (e.g. a FACSVantage™ Cell Sorter, Becton Dickinson Immunocytometry Systems, San Jose, CA) to sort and collect transduced cells expressing a selectable cell surface marker. Following transduction, the cells are stained with fluorescent-labeled antibody molecules directed against the particular cell surface marker. The amount of bound antibody on each cell can be measured by passing droplets containing the cells through the cell sorter. By imparting an electromagnetic charge to droplets containing the stained cells, the transduced cells can be separated from other cells. The positively selected cells are then harvested in sterile collection vessels. These cell sorting procedures are described in detail, for example, in the FACSVantage™ Training Manual, with particular reference to sections 3-11 to 3-28 and 10-1 to 10-17.

Positive selection of the transduced cells can also be performed using magnetic separation of cells based on expression of a particular cell surface marker. In such

separation techniques, cells to be positively selected are first contacted with specific binding agent (e.g., an antibody or reagent that interacts specifically with the cell surface marker). The cells are then contacted with
5 retrievable particles (e.g., magnetically responsive particles) which are coupled with a reagent that binds the specific binding agent (that has bound to the positive cells). The cell-binding agent-particle complex can then be physically separated from non-labeled cells,
10 for example using a magnetic field. When using magnetically responsive particles, the labeled cells can be retained in a container using a magnetic field while the negative cells are removed. These and similar separation procedures are known to those of ordinary
15 skill in the art.

Expression of the vector in the selected transduced cells can be assessed by a number of assays known to those skilled in the art. For example, Western blot or Northern analysis can be employed depending on the nature
20 of the inserted nucleotide sequence of interest. Once expression has been established and the transformed T cells have been tested for the presence of the selected synthetic expression cassette, they are ready for infusion into a patient via the peripheral blood stream.

25 The invention includes a kit for genetic modification of an ex vivo population of primary mammalian cells. The kit typically contains a gene transfer vector coding for at least one selectable marker and at least one synthetic expression cassette contained
30 in one or more containers, ancillary reagents or hardware, and instructions for use of the kit.

EXPERIMENTAL

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not
5 intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should,
10 of course, be allowed for.

Example 1Generation of Synthetic Gag and Env Expression Cassettes

15 A. Modification of HIV-1 Gag, Gag-protease, Gag-reverse transcriptase and Gag-polymerase Nucleic Acid Coding Sequences

The Gag (SEQ ID NO:1), Gag-protease (SEQ ID NO:2), Gag-polymerase (SEQ ID NO:3), and Gag-reverse
20 transcriptase (SEQ ID NO:77) coding sequences were selected from the HIV-1SF2 strain (Sanchez-Pescador, R., et al., Science 227(4686): 484-492, 1985; Luciw, P.A., et al. U.S. Patent No. 5,156,949, issued October 20, 1992; Luciw, P.A., et al., U.S. Patent No. 5,688,688, November
25 18, 1997). These sequences were manipulated to maximize expression of their gene products.

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human
30 genes. The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a high AU content in the RNA and in a decreased translation ability and instability of the

mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Gag-encoding sequences were modified to be comparable to codon usage found in highly expressed human genes.

5 Figure 11 presents a comparison of the percent A-T content for the cDNAs of stable versus unstable RNAs (comparison window size = 50). Human IFN γ mRNA is known to (i) be unstable, (ii) have a short half-life, and (iii) have a high A-U content. Human GAPDH
10 (glyceraldehyde-3-phosphate dehydrogenase) mRNA is known to (i) be a stable RNA, and (i) have a low A-U content. In Figure 11, the percent A-T content of these two sequences are compared to the percent A-T content of native HIV-1SF2 Gag cDNA and to the synthetic Gag cDNA
15 sequence of the present invention. The top two panels of the figure show the percent A-T content over the length of the sequences for IFN γ and native Gag. The bottom two panels of the figure show the percent A-T content over the length of the sequences for GAPDH and the synthetic
20 Gag. Experiments performed in support of the present invention showed that the synthetic Gag sequences were capable of higher level of protein production (see the Examples) than the native Gag sequences. The data in Figure 11 suggest that one reason for this increased
25 production may be increased stability of the mRNA corresponding to the synthetic Gag coding sequences versus the mRNA corresponding to the native Gag coding sequences.

 Second, there are inhibitory (or instability)
30 elements (INS) located within the coding sequences of the Gag and Gag-protease coding sequences (Schneider R, et al., *J Virol.* 71(7):4892-4903, 1997). RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating

effects of the INS. To overcome the requirement for post-transcriptional activating mechanisms of RRE and Rev, and to enhance independent expression of the Gag polypeptide, the INS were inactivated by introducing multiple point mutations that did not alter the reading frame of the encoded proteins. Figure 1 shows the original SF2 Gag sequence, the location of the INS sequences, and the modifications made to the INS sequences to reduce their effects.

For the Gag-protease sequence (wild type, SEQ ID NO:2; synthetic, SEQ ID NOs:5, 78 and 79), the changes in codon usage were restricted to the regions up to the -1 frameshift and starting again at the end of the Gag reading frame (Figure 2; the region indicated in lower case letters in Figure 2 is the unmodified region). Further, inhibitory (or instability) elements (INS) located within the coding sequences of the Gag-protease polypeptide coding sequence were altered as well (indicated in Figure 2). The synthetic coding sequences were assembled by the Midland Certified Reagent Company (Midland, Texas).

Modification of the Gag-polymerase sequences (wild type, SEQ ID NO:3; synthetic, SEQ ID NO:6) and Gag-reverse transcriptase sequences (SEQ ID NOs:80 through 84) include similar modifications as described for Gag-protease in order to preserve the frameshift region. Locations of the inactivation sites and changes to the sequence to alter the inactivation sites are presented in Figure 12 for the native HIV-1_{SF2} Gag-polymerase sequence.

In one embodiment of the invention, the full length polymerase coding region of the Gag-polymerase sequence is included with the synthetic Gag sequences in order to increase the number of epitopes for virus-like particles expressed by the synthetic, optimized Gag expression

cassette. Because synthetic HIV-1 Gag-polymerase expresses the potentially deleterious functional enzymes reverse transcriptase (RT) and integrase (INT) (in addition to the structural proteins and protease), it is important to inactivate RT and INT functions. Several in-frame deletions in the RT and INT reading frame can be made to achieve catalytic nonfunctional enzymes with respect to their RT and INT activity. {Jay. A. Levy (Editor) (1995) *The Retroviridae*, Plenum Press, New York. ISBN 0-306-45033X. Pages 215-20; Grimison, B. and Laurence, J. (1995), *Journal Of Acquired Immune Deficiency Syndromes and Human Retrovirology* 9(1):58-68; Wakefield, J. K., et al., (1992) *Journal Of Virology* 66(11):6806-6812; Esnouf, R., et al., (1995) *Nature Structural Biology* 2(4):303-308; Maignan, S., et al., (1998) *Journal Of Molecular Biology* 282(2):359-368; Katz, R. A. and Skalka, A. M. (1994) *Annual Review Of Biochemistry* 73 (1994); Jacobo-Molina, A., et al., (1993) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 90(13):6320-6324; Hickman, A. B., et al., (1994) *Journal Of Biological Chemistry* 269(46):29279-29287; Goldgur, Y., et al., (1998) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 95(16):9150-9154; Goette, M., et al., (1998) *Journal Of Biological Chemistry* 273(17):10139-10146; Gorton, J. L., et al., (1998) *Journal of Virology* 72(6):5046-5055; Engelman, A., et al., (1997) *Journal Of Virology* 71(5):3507-3514; Dyda, F., et al., *Science* 266(5193):1981-1986; Davies, J. F., et al., (1991) *Science* 252(5002):88-95; Bujacz, G., et al., (1996) *Febs Letters* 398(2-3):175-178; Beard, W. A., et al., (1996) *Journal Of Biological Chemistry* 271(21):12213-12220; Kohlstaedt, L. A., et al., (1992)

Science 256(5065):1783-1790; Krug, M. S. and Berger, S. L. (1991) *Biochemistry* 30(44):10614-10623; Mazumder, A., et al., (1996) *Molecular Pharmacology* 49(4):621-628; Palaniappan, C., et al., (1997) *Journal Of Biological Chemistry* 272(17):11157-11164; Rodgers, D. W., et al., (1995) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 92(4):1222-1226; Sheng, N. and Dennis, D. (1993) *Biochemistry* 32(18):4938-4942; Spence, R. A., et al., (1995) *Science* 267(5200):988-993.)

10 Furthermore selected B- and/or T-cell epitopes can be added to the Gag-polymerase constructs within the deletions of the RT- and INT-coding sequence to replace and augment any epitopes deleted by the functional modifications of RT and INT. Alternately, selected B- and T-cell epitopes (including CTL epitopes) from RT and INT can be included in a minimal VLP formed by expression of the synthetic Gag or synthetic GagProt cassette, described above. (For descriptions of known HIV B- and T-cell epitopes see, HIV Molecular Immunology Database CTL Search Interface; Los Alamos Sequence Compendia, 1987-1997; Internet address: <http://hiv-web.lanl.gov/immunology/index.html>.)

20 The resulting modified coding sequences are presented as a synthetic Gag expression cassette (SEQ ID NO:4), a synthetic Gag-protease expression cassette (SEQ ID NOs:5, 78 and 79), and a synthetic Gag-polymerase expression cassette (SEQ ID NO:6). Synthetic expression cassettes containing codon modifications in the reverse transcriptase region are shown in SEQ ID NOs:80 through 84. An alignment of selected sequences is presented in Figure 7. A common region (Gag-common; SEQ ID NO:9) extends from position 1 to position 1262.

30 The synthetic DNA fragments for Gag and Gag-protease were cloned into the following expression vectors:

pCMVKm2, for transient expression assays and DNA immunization studies, the pCMVKm2 vector was derived from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986) and comprises a kanamycin selectable marker, a
5 ColE1 origin of replication, a CMV promoter enhancer and Intron A, followed by an insertion site for the synthetic sequences described below followed by a polyadenylation signal derived from bovine growth hormone -- the pCMVKm2 vector differs from the pCMV-link vector only in that a
10 polylinker site was inserted into pCMVKm2 to generate pCMV-link (Figure 14, polylinker at positions 1646 to 1697); pESN2dhfr (Figure 13A) and pCMVPLEdhfr (also known as pCMVIII as shown in Figure 13B), for expression in Chinese Hamster Ovary (CHO) cells; and, pAcC13, a shuttle
15 vector for use in the Baculovirus expression system (pAcC13, was derived from pAcC12 which was described by Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990).

A restriction map for vector pCMV-link is presented
20 in Figure 14. In the figure, the CMV promoter (CMV IE ENH/PRO), bovine growth hormone terminator (BGH pA), kanamycin selectable marker (kan), and a ColE1 origin of replication (ColE1 ori) are indicated. A polycloning site is also indicated in the figure following the CMV
25 promoter sequences.

A restriction map for vector pESN2dhfr is presented
in Figure 13A. In the figure, the CMV promoter (pCMV, hCMVIE), bovine growth hormone terminator (BGHpA), SV40
origin of replication (SV40ori), neomycin selectable
30 marker (Neo), SV40 polyA (SV40pA), Adenovirus 2 late promoter (Ad2VLP), and the murine dhfr gene (mu dhfr) are indicated. A polycloning site is also indicated in the figure following the CMV promoter sequences.

Briefly, construction of pCMVPLEdhfr (pCMVIII) was as follows. To construct a DHFR cassette, the EMCV IRES (internal ribosome entry site) leader was PCR-amplified from pCite-4a+ (Novagen, Inc., Milwaukee, WI) and
5 inserted into pET-23d (Novagen, Inc., Milwaukee, WI) as an Xba-Nco fragment to give pET-EMCV. The dhfr gene was PCR-amplified from pESN2dhfr to give a product with a Gly-Gly-Gly-Ser spacer in place of the translation stop codon and inserted as an Nco-BamH1 fragment to give pET-
10 E-DHFR. Next, the attenuated neo gene was PCR amplified from a pSV2Neo (Clontech, Palo Alto, CA) derivative and inserted into the unique BamH1 site of pET-E-DHFR to give pET-E-DHFR/Neo_(m2). Then, the bovine growth hormone terminator from pCDNA3 (Invitrogen, Inc., Carlsbad, CA)
15 was inserted downstream of the neo gene to give pET-E-DHFR/Neo_(m2)BGht. The EMCV-dhfr/neo selectable marker cassette fragment was prepared by cleavage of pET-E-DHFR/Neo_(m2)BGht. The CMV enhancer/promoter plus Intron A was transferred from pCMV6a (Chapman et al., Nuc. Acids
20 Res. (1991) 19:3979-3986) as a HindIII-SalI fragment into pUC19 (New England Biolabs, Inc., Beverly, MA). The vector backbone of pUC19 was deleted from the NdeI to the SapI sites. The above described DHFR cassette was added to the construct such that the EMCV IRES followed the CMV
25 promoter to produce the final construct. The vector also contained an amp^r gene and an SV40 origin of replication.

Selected pCMVKm2 vectors containing the synthetic expression cassettes have been designated as follows: pCMVKm2.GagMod.SF2, pCMVKm2.GagprotMod.SF2, and
30 pCMVKm2.GagpolMod.SF2, pCMVKm2.GagprotMod.SF2.GP1 (SEQ ID NO:78) and pCMVKm2.GagprotMod.SF2.GP2 (SEQ ID NO:79). Other exemplary Gag-encoding expressing cassettes are shown in the Figures and as Sequence Listings.

B. Modification of HIV-1 Gag/Hepatitis C Core Chimeric Protein Nucleic Acid Coding Sequences Generation of Synthetic Expression Cassettes

To facilitate the ligation of the Gag and HCV core coding sequences, PCR amplification was employed. The synthetic p55Gag expression cassette was used as a PCR template with the following primers: GAG5 (SEQ ID NO:11) and P55-SAL3 (SEQ ID NO:12). The PCR amplification was conducted at 55°C for 25 cycles using Stratagene's Pfu polymerase. The resulting PCR product was rendered free of nucleotides and primers using the Promega PCR clean-up kit and then subjected to EcoRI and SalI digestions. For HCV core coding sequences, the following primers were used with an HCV template (Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton, M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997): CORESAL 5 (SEQ ID NO:13) and 173CORE (SEQ ID NO:14) using the conditions outlined above. The purified product was digested with SalI and BamHI restriction enzymes. The digested Gag and HCV core PCR products were ligated into the pCMVKm2 vector digested with EcoRI and BamHI. Ligation of the PCR products at the SalI site resulted in a direct fusion of the final amino acid of p55Gag to the second amino acid of HCV core, serine. Amino acid 173 of core is a serine and is followed immediately by a TAG termination codon. The sequence of the fusion clone was confirmed. The pCMVKm2 vector containing the synthetic expression

cassette was designated as pCMVKm2.GagModHCVcore.

The EcoRI-BamHI fragment of p55Gag-core 173 was also cloned into EcoRI-BamHI-digested pAcC13 for baculovirus expression. Western blots confirmed expression and sucrose gradient sedimentation along with electron microscopy confirmed particle formation. To generate the above clone but containing the synthetic Gag sequences (instead of wild-type), the following steps were performed: pCMVKm2-modified p55Gag was used as template for PCR amplification with MS65 (SEQ ID NO:15) and MS66 (SEQ ID NO:16) primers. The region amplified corresponds to the BspHI and SalI sites at the C-terminus of synthetic Gag sequence. The amplification product was digested with BspHI and SalI and ligated to SalI/BamHI digested pCMV-link along with the Sal/BspHI fragment from pCMV-Km-p55modGag, representing the amino terminal end of modified Gag, and the SalI/BamHI fragment from pCMV-p55Gag-core173. Thereafter, a T4-blunted-SalI partial/BamHI fragment was ligated into pAcC4-SmaI/BamHI to generate pAcC4-p55GagMod-core173 (containing the synthetic sequence presented as SEQ ID NO:7).

C. Defining of the Major Homology Region (MHR) of HIV-1 p55Gag

The Major Homology Region (MHR) of HIV-1 p55 (Gag) is located in the p24-CA sequence of Gag. It is a conserved stretch of 20 amino acids (SEQ ID NO:19). The position in the wild type HIV-1_{SF2} Gag protein is from aa 286-305 and spans a region from nucleotides 856-915 in the native HIV-1_{SF2} Gag DNA-sequence. The position in the synthetic Gag protein is from aa 288-307 and spans a region from nucleotides 862-921 for the synthetic Gag DNA-sequence. The nucleotide sequence for the MHR in the synthetic

GagMod.SF2 is presented as SEQ ID NO:20. Mutations or deletions in the amino acid sequence of the MHR can severely impair particle production (Borsetti, A., et al., *J. Virol.* 72(11):9313-9317, 1998; Mammano, F., et al., *J Virol* 68(8):4927-4936, 1994).

Percent identity to the MHR nucleotide sequence can be determined, for example, using the MacDNAsis program (Hitachi Software Engineering America Limited, South San Francisco, CA), Higgins algorithm, with the following exemplary parameters: gap penalty = 5, no. of top diagonals = 5, fixed gap penalty = 5, K-tuple = 2, window size = 5, and floating gap penalty = 10.

D. Generation of Synthetic Env Expression Cassettes

Env coding sequences of the present invention include, but are not limited to, polynucleotide sequences encoding the following HIV-encoded polypeptides: gp160, gp140, and gp120 (see, e.g., U.S. Patent No. 5,792,459 for a description of the HIV-1_{SF2} ("SF2") Env polypeptide). The relationships between these polypeptides is shown schematically in Figure 15 (in the figure: the polypeptides are indicated as lines, the amino and carboxy termini are indicated on the gp160 line; the open circle represents the oligomerization domain; the open square represents a transmembrane spanning domain (TM); and "c" represents the location of a cleavage site, in gp140.mut the "X" indicates that the cleavage site has been mutated such that it no longer functions as a cleavage site). The polypeptide gp160 includes the coding sequences for gp120 and gp41. The polypeptide gp41 is comprised of several domains including an oligomerization domain (OD) and a transmembrane spanning domain (TM). In the native envelope, the oligomerization domain is required for the

non-covalent association of three gp41 polypeptides to form a trimeric structure: through non-covalent interactions with the gp41 trimer (and itself), the gp120 polypeptides are also organized in a trimeric structure.

5 A cleavage site (or cleavage sites) exists approximately between the polypeptide sequences for gp120 and the polypeptide sequences corresponding to gp41. This cleavage site(s) can be mutated to prevent cleavage at the site. The resulting gp140 polypeptide corresponds to

10 a truncated form of gp160 where the transmembrane spanning domain of gp41 has been deleted. This gp140 polypeptide can exist in both monomeric and oligomeric (i.e. trimeric) forms by virtue of the presence of the oligomerization domain in the gp41 moiety. In the

15 situation where the cleavage site has been mutated to prevent cleavage and the transmembrane portion of gp41 has been deleted the resulting polypeptide product is designated "mutated" gp140 (e.g., gp140.mut). As will be apparent to those in the field, the cleavage site can be

20 mutated in a variety of ways. The native amino acid sequence in the SF162 cleavage sites is: APTKAKRRVVQREKR (SEQ ID NO:21), where KAKRR (SEQ ID NO:22) is termed the "second" site and REKR (SEQ ID NO:23) is the "first site". Exemplary mutations include the following

25 constructs: gp140.mut7.modSF162 which encodes the amino acid sequence APTKA**I**SSVVQSEKS (SEQ ID NO:24) in the cleavage site region; gp140.mut8.modSF162 which encodes the amino acid sequence APT**I**A**I**SSVVQSEKS (SEQ ID NO:25) in the cleavage site region and gp140mut.modSF162 which

30 encodes the amino acid sequence APTKAKRRVVQREKS (SEQ ID NO:26). Mutations are denoted in bold. The native amino acid sequence in the US4 cleavage sites is: APTQAKRRVVQREKR (SEQ ID NO:27), where QAKRR (SEQ ID NO:28) is termed the "second" site and REKR (SEQ ID

NO:23) is the "first site". Exemplary mutations include the following construct: gp140.mut.modUS4 which encodes the amino acid sequence APTQAKRRVVQREKS (SEQ ID NO:29) in the cleavage site region. Mutations are denoted in bold.

5

E. Modification of HIV-1 Env (Envelope) Nucleic Acid Coding Sequences

In one embodiment of the present invention, wild-type Env coding sequences were selected from the HIV-1^{SF162} ("SF162") strain (Cheng-Mayer (1989) PNAS USA 86:8575-8579). These SF162 sequences were as follows: gp120, SEQ ID NO:30 (Fig. 16); gp140, SEQ ID NO:31 (Fig. 17); and gp160, SEQ ID NO:32 (Fig. 18).

In another embodiment of the present invention, wild-type Env coding sequences were selected from the HIV-US4 strain (Mascola, et al. (1994) *J. Infect. Dis.* 169:48-54). These US4 sequences were as follows: gp120, SEQ ID NO:51 (Fig. 38); gp140, SEQ ID NO:52 (Fig. 39); and gp160, SEQ ID NO:53 (Fig. 40).

These Env coding sequences were manipulated to maximize expression of their gene products.

First, the wild-type coding region was modified in one or more of the following ways. In one embodiment, sequences encoding hypervariable regions of Env, particularly V1 and/or V2 were deleted. In other embodiments, mutations were introduced into sequences encoding the cleavage site in Env to abrogate the enzymatic cleavage of oligomeric gp140 into gp120 monomers. (See, e.g., Earl et al. (1990) PNAS USA 87:648-652; Earl et al. (1991) *J. Virol.* 65:31-41). In yet other embodiments, hypervariable region(s) were deleted, N-glycosylation sites were removed and/or cleavage sites mutated.

Second, the HIV-1 codon usage pattern was modified

so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes. The HIV codon usage reflects a high content of the nucleotides A or T in the codon-triplet. The effect of
5 the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Env coding sequences were modified to be comparable
10 to codon usage found in highly expressed human genes.

Figures 22A-22H present comparisons of the percent A-T content for the cDNAs of stable versus unstable RNAs (comparison window size = 50). Human IFN γ mRNA is known to (i) be unstable, (ii) have a short half-life, and
15 (iii) have a high A-U content. Human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA is known to (i) be a stable RNA, and (i) have a low A-U content. In Figures 22A-H, the percent A-T content of these two sequences are compared to the percent A-T content of (1)
20 native HIV-1 US4 Env gp160 cDNA, a synthetic US4 Env gp160 cDNA sequence (i.e., having modified codons) of the present invention; and (2) native HIV-1 SF162 Env gp160 cDNA, a synthetic SF162 Env gp160 cDNA sequence (i.e., having modified codons) of the present invention.
25 Figures 22A-H show the percent A-T content over the length of the sequences for IFN γ (Figures 22C and 22G); native gp160 Env US4 and SF162 (Figures 22A and 22E, respectively); GAPDH (Figures 22D and 22H); and the synthetic gp160 Env for US4 and SF162 (Figures 22B and
30 22F). Experiments performed in support of the present invention showed that the synthetic Env sequences were capable of higher level of protein production (see the Examples) than the native Env sequences. The data in Figures 22A-H suggest that one reason for this increased

production is increased stability of the mRNA corresponding to the synthetic Env coding sequences versus the mRNA corresponding to the native Env coding sequences.

5 To create the synthetic coding sequences of the present invention the gene cassettes were designed to comprise the entire coding sequence of interest. Synthetic gene cassettes were constructed by oligonucleotide synthesis and PCR amplification to
10 generate gene fragments. Primers were chosen to provide convenient restriction sites for subcloning. The resulting fragments were then ligated to create the entire desired sequence which was then cloned into an appropriate vector. The final synthetic sequences were
15 (i) screened by restriction endonuclease digestion and analysis, (ii) subjected to DNA sequencing in order to confirm that the desired sequence had been obtained and (iii) the identity and integrity of the expressed protein confirmed by SDS-PAGE and Western blotting (See,
20 Examples. The synthetic coding sequences were assembled at Chiron Corp. or by the Midland Certified Reagent Company (Midland, Texas).

Exemplary modified coding sequences are presented as synthetic Env expression cassettes in Table 1A and 1B.
25 The following expression cassettes (i) have unique, terminal *EcoRI* and *XbaI* cloning sites; (ii) include Kozak sequences to promote optimal translation; (iii) tPA signal sequences (to direct the ENV polypeptide to the cell membrane, see, e.g., Chapman et al., *infra*); (iv)
30 open reading frames optimized for expression in mammalian cells; and (v) a translational stop signal codon.

Table 1A: Exemplary Synthetic Env Expression
Cassettes (SF162)

	Expression Cassette	Seq Id	Further Information
5	gp120 SF162	30	wild-type; Figure 16
	gp140 SF162	31	wild-type; Figure 17
	gp160 SF162	32	wild-type; Figure 18
	gp120.modSF162	33	none; Figure 19
	gp120.modSF162.delV2	34	deleted V2 loop; Figure 20
10	gp120.modSF162.delV1/V2	35	deleted V1 and V2; Figure 21
	gp140.modSF162	36	none; Figure 23
	gp140.modSF162.delV2	37	deleted V2 loop; Figure 24
	gp140.modSF162.delV1/V2	38	deleted V1 and V2; Figure 25
	gp140.mut.modSF162	39	mutated cleavage site; Fig. 26
15	gp140.mut.modSF162.delV2	40	deleted V2; mutated cleavage site; Figure 27
	gp140.mut.modSF162.delV1/V 2	41	deleted V1 & V2; mutated cleavage site; Figure 28
	gp140.mut7.modSF162	42	mutated cleavage site; Fig. 29
	gp140.mut7.modSF162.delV2	43	mutated cleavage site; deleted V2; Figure 30
20	gp140.mut7.modSF162.delV1/ V2	44	mutated cleavage site; deleted V1 and V2; Figure 31
	gp140.mut8.modSF162	45	mutated cleavage site; Fig. 32
	gp140.mut8.modSF162.delV2	46	mutated cleavage site; deleted V2; Figure 33
25	gp140.mut8.modSF162.delV1/ V2	47	mutated cleavage site; deleted V1 and V2; Figure 34
	gp160.modSF162	48	none; Figure 35
	gp160.modSF162.delV2	49	deleted V2 loop; Figure 36
	gp160.modSF162.delV1/V2	50	deleted V1 & V2; Figure 37

Table 1B:
Exemplary Synthetic Env Expression Cassettes (US4)

Expression Cassette	Seq Id	Further Information
gp120 US4	51	wild-type; Figure 38
gp140 US4	52	wild-type; Figure 39
gp160 US4	53	wild-type; Figure 40
gp120.modUS4	54	none; Figure 41
gp120.modUS4.del 128-194	55	deletion in V1 and V2 regions; Figure 42
gp140.modUS4	56	none; Figure 43
gp140.mut.modUS4	57	mutated cleavage site; Figure 44
gp140TM.modUS4	58	native transmembrane region; Figure 45
gp140.modUS4.delV1/V2	59	deleted V1 and V2; Figure 46
gp140.modUS4.delV2	60	deleted V1; Figure 47
gp140.mut.modUS4.delV1/V2	61	mutated cleavage site; deleted V1 and V2; Figure 48
gp140.modUS4.del 128-194	62	deletion in V1 and V2 regions; Figure 49
gp140.mut.modUS4.del 128- 194	63	mutated cleavage site; deletion in V1 and V2 regions; Figure 50
gp160.modUS4	64	none; Figure 51
gp160.modUS4.delV1	65	deleted V1; Figure 52
gp160.modUS4.delV2	66	deleted V2; Figure 53
gp160.modUS4.delV1/V2	67	deleted V1 and V2; Figure 54
gp160.modUS4del 128-194	68	deletion in V1 and V2 regions; Figure 55

Alignments of the sequences presented in the above
tables are presented in Figures 66A and 66B.

A common region (Env-common) extends from nucleotide
position 1186 to nucleotide position 1329 (SEQ ID NO:69,

Fig. 56) relative to the wild-type US4 sequence and from nucleotide position 1117 to position 1260 (SEQ ID NO:79, Fig. 57) relative to the wild-type SF162 sequence. The synthetic sequences of the present invention

5 corresponding to these regions are presented, as SEQ ID NO:71 (Figure 58) for the synthetic Env US4 common region and as SEQ ID NO:72 (Figure 59) for the synthetic Env SF162 common region.

Percent identity to this sequence can be determined, 10 for example, using the Smith-Waterman search algorithm (Time Logic, Incline Village, NV), with the following exemplary parameters: weight matrix = nuc4x4hb; gap opening penalty = 20, gap extension penalty = 5, reporting threshold = 1; alignment threshold = 20.

15 Various forms of the different embodiments of the present invention (e.g., constructs) may be combined.

F. Cloning Synthetic Env Expression Cassettes of the Present Invention.

20 The synthetic DNA fragments encoding the Env polypeptides were typically cloned into the eucaryotic expression vectors described above for Gag, for example, pCMVKm2/pCMVlink (Figure 4), pCMV6a, pESN2dhfr (Figure 13A), pCMVIII (Figure 13B; alternately designated as the 25 pCMV-PL-E-dhfr/neo vector).

Exemplary designations for pCMVlink vectors containing synthetic expression cassettes of the present invention are as follows: pCMVlink.gp140.modSF162; pCMVlink.gp140.-modSF162.delV2; 30 pCMVlink.gp140.mut.modSF162; pCMVlink.gp140.mut.modSF162.delV2; pCMVKm2.gp140modUS4; pCMVKm2.gp140.modUS4.delV2; pCMVKm2.gp140.mut.modUS4; and, pCMVKm2.gp140.mut.modUS4.delV1/V2.

G. Generation of Synthetic Tat Expression Cassettes

Tat coding sequences have also been modified according to the teachings of the present specification. The wild type nucleotide sequence encoding tat from variant SF162 is presented in Figure 76 (SEQ ID NO:85). The corresponding wild-type amino acid sequence is presented in Figure 77 (SEQ ID NO:86). Figure 81 (SEQ ID NO:89) shows the nucleotide sequence encoding the amino terminal of the tat protein and the codon encoding cystein-22 is underlined. Other exemplary constructs encoding synthetic tat polypeptides are shown in Figures 78 and 79 (SEQ ID NOs:87 and 88). In one embodiment (SEQ ID NO:88), the cystein residue at position 22 is replaced by a glycine. Caputo et al. (1996) *Gene Therapy* 3:235 have shown that this mutation affects the trans activation domain of Tat.

Various forms of the different embodiments of the invention, described herein, may be combined.

H. Deposit of Vectors

Selected exemplary constructs shown below and described herein are deposited at Chiron Corporation, Emeryville, CA, 94662-8097, and were sent to the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on December 27, 1999.

	Plasmid Name	Chiron Deposit #	Date Sent to ATCC
	pCMVgp160.modUS4	5094	27 Dec 99
	pCMVgp160del1.modUS4	5095	27 Dec 99
	pCMVgp160del2.modUS4	5096	27 Dec 99
5	pCMVgp160del-2.modUS4	5097	27 Dec 99
	pCMVgp160del128-194.mod.US4	5098	27 Dec 99
	pCMVgp140mut.modUS4del128-194	5100	27 Dec 99
	pCMVgp140.mut.mod.US	5101	27 Dec 99
	pCMVgp160.modSF162	5125	27 Dec 99
10	pCMVgp160.modSF162.delV2	5126	27 Dec 99
	pCMVgp160.modSF162.delV1V2	5127	27 Dec 99
	pCMVgp140.mut.modSF162delV2	5128	27 Dec 99
	pCMVgp140.mut7.modSF162	5129	27 Dec 99
	pCMVgp140.mut7.modSF162delV2	5130	27 Dec 99
15	pCMVgp140.mut8.modSF162	5131	27 Dec 99
	pCMVgp140.mut8.modSF162delV2	5132	27 Dec 99
	pCMVgp140.mut8.modSF162delV1V2	5133	27 Dec 99
	pCMVKm2.Gagprot.Mod.SF2.GP1	5150	27 Dec 99
20	pCMVKm2.Gagprot.Mod.SF2.GP2	5151	27 Dec 99

Example 2Expression Assays for theSynthetic Gag, Env and Tat Coding Sequences25 A. Gag and Gag-Protease Coding Sequences

The HIV-1SF2 wild-type Gag (SEQ ID NO:1) and Gag-protease (SEQ ID NO:2) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Gag (SEQ ID NO:4) and

30 Gag-protease (SEQ ID NOs:5, 78 or 79)) sequences were cloned.

Expression efficiencies for various vectors carrying the HIV-1SF2 wild-type and synthetic Gag sequences were evaluated as follows. Cells from several mammalian cell lines (293, RD, COS-7, and CHO; all obtained from the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209) were transfected with 2 μ g of DNA in transfection reagent LT1 (PanVera Corporation, 545 Science Dr., Madison, WI). The cells were incubated for 5 hours in reduced serum medium (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium was then replaced with normal medium as follows: 293 cells, IMDM, 10% fetal calf serum, 2% glutamine (BioWhittaker, Walkersville, MD); RD and COS-7 cells, D-MEM, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD); and CHO cells, Ham's F-12, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The cells were incubated for either 48 or 60 hours. Supernatants were harvested and filtered through 0.45 μ m syringe filters and, optionally, stored at -20°C.

Supernatants were evaluated using the Coulter p24-assay (Coulter Corporation, Hialeah, FL, US), using 96-well plates coated with a murine monoclonal antibody directed against HIV core antigen. The HIV-1 p24 antigen binds to the coated wells. Biotinylated antibodies against HIV recognize the bound p24 antigen. Conjugated strepavidin-horseradish peroxidase reacts with the biotin. Color develops from the reaction of peroxidase with TMB substrate. The reaction is terminated by addition of 4N H₂SO₄. The intensity of the color is directly proportional to the amount of HIV p24 antigen in a sample.

The results of these expression assays are presented in Tables 2A and 2B. Tables 2A and 2B shows data

obtained using the synthetic Gag-protease expression cassette of SEQ ID NO:5. Similar results were obtained using the Gag-protease expression cassettes of SEQ ID NOs:78 and 79.

5

Table 2: in vitro gag and gagprot p24 expression

5 TABLE 2a. Increased in vitro expression from modified vs. native gag plasmids in supernatants and lysates from transiently transfected cells

experiment	native (nat) ^a modified (mod) ^b	supernatant (sup) lysate (lys)	cell line	hours post transfection	total ng p24 (fold increase)
1	nat	sup	293	48	3.4
	mod	sup	293	48	1260 (371)
	nat	sup	293	60	3.2
	mod	sup	293	60	2222 (694)
2	nat	sup	293	60	1.8
	mod	sup	293	60	1740 (966)
3	nat	sup	293	60	1.8
	mod	sup	293	60	580 (322)
4	nat	lys	293	60	1.5
	mod	lys	293	60	85 (57)
1	nat	sup	RD	48	5.6
	mod	sup	RD	48	66 (12)
	nat	sup	RD	60	7.8
	mod	sup	RD	60	70.2 (9)
2	nat	lys	RD	60	1.9
	mod	lys	RD	60	7.8 (4)
1	nat	sup	COS-7	48	0.4
	mod	sup	COS-7	48	33.4 (84)
2	nat	sup	COS-7	48	0.4
	mod	sup	COS-7	48	10 (25)
	nat	lys	COS-7	48	3
	mod	lys	COS-7	48	14 (5)

^a pCMVLink.Gag.SF2.PRE

^b pCMVKm2.GagMod.SF2

5 TABLE 2b. In vitro expression from modified gag and gagprotease plasmids in supernatants and lysates from transiently transfected cells

plasmid	supernatant (sup) lysate (lys)	cell line	hours post transfection	total ng p24 ^d
Gag ^a	sup	293	60	760
GagProt(GP1) ^b	sup	293	60	380
GagProt(GP2) ^c	sup	293	60	320
Gag	lys	293	60	78
GagProt(GP1)	lys	293	60	1250
GagProt(GP2)	lys	293	60	400
Gag	sup	COS-7	72	40
GagProt(GP1)	sup	COS-7	72	150
GagProt(GP2)	sup	COS-7	72	290
Gag	lys	COS-7	72	60
GagProt(GP1)	lys	COS-7	72	63
GagProt(GP2)	lys	COS-7	72	58

^a pCMVKm2.GagMod.SF2

^b pCMVKm2.GagProtMod.SF2 (GP1) gagprotease with codon optimization and inactivation of INS in protease

^c pCMVKm2.GagProtMod.SF2 (GP2) gagprotease with only inactivation of INS in protease

^d Shown are representative results from 3 independent experiments for each cell line tested.

The data showed that the synthetic Gag and Gag-protease expression cassettes provided dramatic increases in production of their protein products, relative to the native (HIV-1SF2 wild-type) sequences, when expressed in a variety of cell lines.

B. Env Coding Sequences

The HIV-SF162 ("SF162") wild-type Env (SEQ ID NO:1-3) and HIV-US4 ("US4") wild-type Env (SEQ ID NO:22-24) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Env sequences were cloned.

Expression efficiencies for various vectors carrying the SF162 and US4 wild-type and synthetic Env sequences were evaluated essentially as described above for Gag except that cell lysates were prepared in 40 μ l lysis buffer (1.0 % NP40, 0.1 M Tris pH 7.5) and frozen at -20°C and capture ELISAs were performed as follows.

For Capture ELISAs, 250 ng of an ammonium sulfate IgG cut of goat polyclonal antibody to gp120SF2/env2-3 was used to coat each well of a 96-well plate (Corning, Corning, NY). Serial dilutions of gp120/SF2 protein (MID 167) were used to set the quantitation curve from which expression of US4 or SF162 gp120 proteins from transfection supernatant and lysates were calculated. Samples were screened undiluted and, optionally, by serial 2-fold dilutions. A human polyclonal antibody to HIV-1 gp120/SF2 was used to detect bound gp120 envelope protein, followed by horse-radish peroxidase (HRP)-labeled goat anti-human IgG conjugates. TMB (Pierce, Rockford, IL) was used as the substrate and the reaction is terminated by addition of 4N H₂SO₄. The reaction was quantified by measuring the optical density (OD) at 450 nm. The intensity of the color is directly

proportional to the amount of HIV gp120 antigen in a sample. Purified SF2 gp120 protein was diluted and used as a standard.

5 The results of the transient expression assays are presented in Tables 3 and 4. Table 3 depicts transient expression in 293 cells transfected with a pCMVKm2 vector carrying the Env cassette of interest. Table 4 depicts transient expression in RD cells transfected with a pCMVKm2 vector carrying the Env cassette of interest.

Table 3

Native (N) Synthetic (S)	Cell Line	Total sup (ng)	Sup fold increase (S v. N)	Total cell lysate (ng)	Cell lysate fold increase (S v. N)	Total (ng)	Total fold increase (S v. N)
N-gp120.US4	RD	87		<1		88	
S-gp120.modUS4	RD	690	8	2	5	693	8
N-gp140.US4	RD	526		0		526	
S-gp140.modUS4	RD	1305	2	1	2	1306	2
S-gp140mut.modUS4	RD	35	N/A	25	N/A	60	N/A
S-gp140TM.modUS4	RD	0	N/A	5	N/A	5	N/A
N-gp160.US4	RD	0		8		8	
S-gp160.modUS4	RD	0	0	30	4	30	4

Table 4

CHO Cell Lines Expression Level of US4 Envelope Constructs			
Constructs	CHO Clone #	MTX Level	Expression Level* (ng/ml)
gp120.modUS4	1	3.2 μ M	250-450
	2	1.6 μ M	350-450
	3	200nM	230-580
	4	200nM	300-500
gp140.modUS4	1	1 μ M	155-300
	2	1 μ M	100-260
	3	1 μ M	200-430
gp140.mut. modUS4	1	1 μ M	110-270
	2	1 μ M	100-235
	3	1 μ M	100-220
gp140.modUS4 .delV1/V2	1	50nM	313-587**
	2	50nM	237-667**
	3	50nM	492-527**
gp140.mut. modUS4.delV1 /V2	1	50nM	46-328**
	2	50nM	82-318**
	3	50nM	204-385**

*All samples measured at T-75 flask stage unless otherwise indicated

**at 24 well and 6 well plate stages

***in a three liter bioreactor perfusion culture this clone yielded approximately 2-5 μ g/ml.

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The data showed that the synthetic Env and expression cassettes provided a significant increase in production of their protein products, relative to the native (HIV-1SF162 or US4 wild-type) sequences, when
5 expressed in a variety of cell lines.

C. CHO Cell line Env expression data

Chinese hamster ovary (CHO) cells were transfected with plasmid DNA encoding the synthetic HIV-1 gp120 or
10 gp140 proteins (e.g., pESN2dhfr or pCMVIII vector backbone) using Mirus TransIT-LT1 polyamine transfection reagent (Pan Vera) according to the manufacturers instructions and incubated for 96 hours. After 96 hours, media was changed to selective media (F12 special with
15 250 µg/ml G418) and cells were split 1:5 and incubated for an additional 48 hours. Media was changed every 5-7 days until colonies started forming at which time the colonies were picked, plated into 96 well plates and screened by gp120 Capture ELISA. Positive clones were
20 expanded in 24 well plates and screened several times for Env protein production by Capture ELISA, as described above. After reaching confluency in 24 well plates, positive clones were expanded to T25 flasks (Corning, Corning, NY). These were screened several times after
25 confluency and positive clones were expanded to T75 flasks.

Positive T75 clones were frozen in LN2 and the highest expressing clones amplified with 0-5 µM methotrexate (MTX) at several concentrations and plated in
30 100mm culture dishes. Plates were screened for colony formation and all positive clones were again expanded as described above. Clones were expanded and amplified and screened at each step by gp120 capture ELISA. Positive clones were frozen at each methotrexate level. Highest

producing clones were grown in perfusion bioreactors (3L, 100L) for expansion and adaptation to low serum suspension culture conditions for scale-up to larger bioreactors.

- 5 Tables 5 and 6 show Capture ELISA data from CHO cells transfected with pCMVIII vector carrying a cassette encoding synthetic HIV-US4 and SF162 Env polypeptides (e.g., mutated cleavage sites, modified codon usage and/or deleted hypervariable regions). Thus, stably
- 10 transfected CHO cell lines which express Env polypeptides (e.g., gp120, gp140-monomeric, and gp140-oligomeric) have been produced.

Table 5

CHO Cell Lines Expression Level of US4 Envelope Constructs			
Constructs	CHO Clone #	MTX Level	Expression Level (ng/ml)
gp120.modUS4	1	3.2 μ M	250-450
	2	1.6 μ M	350-450
	3	200nM	230-580***
	4	200nM	300-500
gp140.modUS4	1	1 μ M	155-300
	2	1 μ M	100-260
	3	1 μ M	200-430
gp140.mut. modUS4	1	1 μ M	110-270
	2	1 μ M	100-235
	3	1 μ M	100-220
gp140.modUS4 .delV1/V2	1	50nM	313-587**
	2	50nM	237-667**
	3	50nM	492-527**
gp140.mut. modUS4.delV1 /V2	1	50nM	46-328**
	2	50nM	82-318**
	3	50nM	204-385**

*All samples measured at T-75 flask stage unless otherwise indicated

**at 24 well and 6 well plate stages

***in a three liter bioreactor perfusion culture this clone yielded approximately 2-5 μ g/ml.

Table 6

CHO Cell Lines Expression Level of SF162 Envelope Constructs			
Constructs	CHO Clone #	MTX Level	Expression Level (ng/ml)
gp120.modSF162	1	0	755-2705
	2	0	928-1538
	3	0	538-1609
gp140.modSF162	1	20 nM	180-350
gp140.mut. modSF162	1	20 nM	164-451
	2	20 nM	188-487
	3	20 nM	233-804
gp120.modSF162 .delV2	1	800nM	528-1560
	2	800nM	487-1878
	3	800nM	589-1212
gp140.modSF162 .delV2	1	800nM	300-600
	2	800nM	200-400
	3	800nM	200-500
gp140.mut. modSF162.delV2	1	800nM	300-700
	2	400nM	1161
	3	800nM	400-600
	4	400nM	1600-2176

*All samples measured at T-75 flask stage unless otherwise indicated

The results presented above demonstrate the ability of the constructs of the present invention to provide expression of Env polypeptides in CHO cells. Production of polypeptides using CHO cells provides (i) correct glycosylation patterns and protein conformation (as determined by binding to panel of MAbs); (ii) correct binding to CD4 receptor molecules; (iii) absence of non-

mammalian cell contaminants (e.g., insect viruses and/or cells); and (iv) ease of purification.

D. Tat Coding Sequences

5 The HIV-SF162 ("SF162") wild-type Tat (SEQ ID NO:85) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Tat sequences were cloned (SEQ ID NOS:87, 88 and 89).

10 Expression efficiencies for various vectors carrying the SF162 wild-type and synthetic Tat sequences are evaluated essentially as described above for Gag and Env using capture ELISAs with the appropriate anti-tat antibodies and/or CHO cell assays. Expression of the polypeptides encoded by the synthetic cassettes is
15 improved relative to wild type.

Example 3

Western Blot Analysis of Expression

A. Gag and Gag-Protease Coding Sequences

20 Human 293 cells were transfected as described in Example 2 with pCMV6a-based vectors containing native or synthetic Gag expression cassettes. Cells were cultivated for 60 hours post-transfection. Supernatants were prepared as described. Cell lysates were prepared
25 as follows. The cells were washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%; Novex, San Diego,
30 CA) were loaded with 20 μ l of supernatant or 12.5 μ l of cell lysate. A protein standard was also loaded (5 μ l, broad size range standard; BioRad Laboratories, Hercules, CA). Electrophoresis was carried out and the proteins were transferred using a BioRad Transfer Chamber (BioRad

Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer (Millipore), where the transfer was performed at 100 volts for 90 minutes. The
5 membranes were exposed to HIV-1-positive human patient serum and immunostained using o-phenylenediamine dihydrochloride (OPD; Sigma).

The results of the immunoblotting analysis showed that cells containing the synthetic Gag expression
10 cassette produced the expected p55 protein at higher per-cell concentrations than cells containing the native expression cassette. The Gag p55 protein was seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants
15 for cells transfected with the synthetic Gag expression cassette of the present invention. Experiments performed in support of the present invention suggest that cells containing the synthetic Gag-prot expression cassette produced the expected Gag-prot protein at comparably
20 higher per-cell concentrations than cells containing the native expression cassette.

In addition, supernatants from the transfected 293 cells were fractionated on sucrose gradients. Aliquots of the supernatant were transferred to Polyclear™ ultra-
25 centrifuge tubes (Beckman Instruments, Columbia, MD), under-laid with a solution of 20% (wt/wt) sucrose, and subjected to 2 hours centrifugation at 28,000 rpm in a Beckman SW28 rotor. The resulting pellet was suspended in PBS and layered onto a 20-60% (wt/wt) sucrose gradient
30 and subjected to 2 hours centrifugation at 40,000 rpm in a Beckman SW41ti rotor.

The gradient was then fractionated into approximately 10 x 1 ml aliquots (starting at the top, 20%-end, of the gradient). Samples were taken from

fractions 1-9 and were electrophoresed on 8-16% SDS polyacrylamide gels. Fraction number 4 (the peak fraction) corresponds to the expected density of Gag protein VLPs. The supernatants from 293/synthetic Gag cells gave much stronger p55 bands than supernatants from 293/native Gag cells, and, as expected, the highest concentration of p55 in either supernatant was found in fraction 4.

These results demonstrate that the synthetic Gag expression cassette provides superior production of both p55 protein and VLPs, relative to the native Gag coding sequences.

B. Env Coding Sequences

Human 293 cells were transfected as described in Example 2 with pCMVKm2-based; pCMVlink-based; p-CMVII-based or pESN2-based vectors containing native or synthetic Env expression cassettes. Cells were cultivated for 48 or 60 hours post-transfection. Cell lysates and supernatants were prepared as described (Example 2). Briefly, the cells were washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO)] in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%; Novex, San Diego, CA) were loaded with 20 μ l of supernatant or 12.5 μ l of cell lysate. A protein molecular weight standard and an HIV SF2 gp120 positive control protein (5 μ l, broad size range standard; BioRad Laboratories, Hercules, CA) were also loaded. Electrophoresis was carried out and the proteins were transferred using a BioRad Transfer Chamber (BioRad Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer

(Millipore), where the transfer was performed at 100 volts for 90 minutes. The membranes were then reacted against polyclonal goat anti-gp120SF2/env2-3 anti-sera, followed by incubation with swine anti-goat IgG-peroxidase (POD) (Sigma, St. Louis, MO). Bands indicative of binding were visualized by adding DAB with hydrogen peroxide which deposits a brown precipitate on the membranes.

The results of the immunoblotting analysis showed that cells containing the synthetic Env expression cassette produced the expected Env gp proteins of the predicted molecular weights as determined by mobilities in SDS-polyacrylamide gels at higher per-cell concentrations than cells containing the native expression cassette. The Env proteins were seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells transfected with the synthetic Env expression cassette of the present invention.

C. Tat Coding Sequences

Human 293 cells are transfected as described in Example 2 with various vectors containing native or synthetic Tat expression cassettes. Cells are cultivated and isolated proteins analyzed as described above. Immunoblotting analysis shows that cells containing the synthetic Tat expression cassette produced the expected Tat proteins of the predicted molecular weights as determined by mobilities in SDS-polyacrylamide gels at higher per-cell concentrations than cells containing the native expression cassette.

Example 4Purification of Env polypeptidesA. Purification of Oligomeric gp140

Purification of oligomeric gp140 (o-gp140 US4) was
5 conducted essentially as shown in Figure 60. For the
experiments described herein, o-gp140 refers to
oligomeric gp140 in either native or modified (e.g.,
optimized expression sequences, deleted, mutated,
truncated, etc.) form. Briefly, concentrated (30-50X)
10 supernatants obtained from CHO cell cultures were loaded
onto an anion exchange (DEAE) column which removed DNA
and other serum proteins. The eluted material was loaded
onto a ceramic hydroxyapatite column (CHAP) which bound
serum proteins but not HIV Env proteins. The flow-
15 through from the DEAE and CHAP columns was loaded onto a
Protein A column as a precautionary step to remove any
remaining serum immunoglobulins. The Env proteins in the
flow-through were then captured using the lectin
gluconan (GNA, Vector Labs, Burlingame, CA).
20 GNA has high affinity for mannose rich carbohydrates such
as Env. The Env proteins were then eluted with GNA
substrate. To remove other highly glycosylated proteins,
a cation exchange column (SP) was used to purify
gp140/gp120. In a final step, which separates gp120 from
25 o-gp140, a gel filtration column was used to separate
oligomers from monomers. Sizing and chromatography
analysis of the final product revealed that this strategy
lead to the successful isolation of oligomeric gp140.

B. Purification of gp120

Purification of gp120 was conducted essentially as
previously described for other Env proteins. Briefly,
concentrated supernatants obtained from CHO cell cultures
were loaded onto an anion exchange (DEAE) column which

removed DNA and other serum proteins. The eluted material was loaded onto a ceramic hydroxyapatite column (CHAP) which bound serum proteins but not HIV Env proteins. The flow-through from the CHAP column was
5 loaded a cation exchange column (SP) where the flow-through was discarded and the bound fraction eluted with salt. The eluted fraction(s) were loaded onto a Suprose 12/Superdex 200 Tandem column (Pharmacia-Upjohn, Uppsala, Sweden) from which purified gp120 was obtained. Sizing
10 and chromatography analysis of the final product revealed that this strategy successfully purified gp120 proteins.

Example 5

Analysis of Purified Env Polypeptides

15 A. Analysis of o-gp140

It is well documented that HIV Env protein binds to CD4 only in its correct conformation. Accordingly, the ability of o-gp140 US4 polypeptides, produced and purified as described above, to bind CD4 cells was
20 tested. O-gp140 US4 was incubated for 15 minutes with FITC-labeled CD4 at room temperature and loaded onto a Biosil 250 (BioRad) size exclusion column using Waters HPLC. CD4-FITC has the longest retention time (2.67 minutes), followed by CD4-FITC-gp120 (2.167 min). The
25 shortest retention time (1.9 min) was observed for CD4-FITC-o-gp140 US4 indicating that, as expected, o-gp140 US4 binds to CD4 forming a large complex which reduces retention time on the column. Thus, the o-gp140 US4 produced and purified as described above is of the
30 correct size and conformation.

In addition, the US4 o-gp140, purified as described above, was also tested for its ability to bind to a variety of monoclonal antibodies with known epitope specificities for the CD4 binding site, the CD4 inducible

site, the V3 loop and oligomer-specific gp41 epitope. O-gp140 bound strongly to these antibodies, indicating that the purified protein retains its structural integrity.

5 B. Analysis of gp120

As described above, CD4-FITC binds gp120, as demonstrated by the decreased retention time on the HPLC column. Thus, US4 gp120 purified by the above method retains its conformational integrity. In addition, the
10 properties of purified gp120 can be tested by examining its integrity and identity on western blots, as well as, by examining protein concentration, pH, conductivity, endotoxin levels, bioburden and the like. US4 gp120, purified as described above, was also tested for its
15 ability to bind to a variety of monoclonal antibodies with known epitope specificities for the CD4 binding site, the CD4 inducible site, the V3 loop and oligomer-specific gp41 epitope. The pattern of mAb binding to gp120 indicated that the purified protein retained its
20 structural integrity, for example, the purified gp120 did not bind the mAb having the oligomer-specific gp41 epitope (as expected).

Example 6

25 Electron Microscopic Evaluation of VLP Production

The cells for electron microscopy were plated at a density of 50-70% confluence, one day before transfection. The cells were transfected with 10 µg of DNA using transfection reagent LT1 (Panvera) and
30 incubated for 5 hours in serum-reduced medium (see Example 2). The medium was then replaced with normal medium (see Example 2) and the cells were incubated for 14 hours (COS-7) or 40 hours (CHO). After incubation the cells were washed twice with PBS and fixed with 2%

glutaraldehyde. Electron microscopy was performed by Prof. T.S. Benedict Yen, Veterans Affairs, Medical Center, San Francisco, CA).

Electron microscopy was carried out using a transmission electron microscope (Zeiss 10c). The cells were pre-stained with osmium and stained with uranium acetate and lead citrate. The magnification was 100,000X.

Figures 3A and 3B show micrographs of CHO cells transfected with pCMVKM2 carrying the synthetic Gag expression cassette (SEQ ID NO:5) or carrying the Gag-prot expression cassette (SEQ ID NO:79). In the figure, free and budding immature virus-like-particles (VLP) of the expected size (100 nm) are seen for the Gag expression cassette (Figure 3A) and both immature and mature VLPs are seen for the Gag-prot expression cassette (Figure 3B). COS-7 cells transfected with the same vector have the same expression pattern. VLP can also be found intracellularly in CHO and COS-7 cells.

Native and synthetic Gag expression cassettes were compared for their associated levels of VLP production when used to transfect human 293 cells. The comparison was performed by density gradient ultracentrifugation of cell supernatants and Western-blot analysis of the gradient fractions. There was a clear improvement in production of VLPs when using the synthetic Gag construct.

Example 7

Expression of Virus-like Particles in the Baculovirus System

A. Expression of Native HIV p55 Gag

To construct the native HIV p55 Gag baculovirus shuttle vector, the prototype SF2 HIV p55 plasmid, pTM1-

Gag (Selby M.J., et al., *J Virol.* 71(10):7827-7831, 1997), was digested with restriction endonucleases *Nco*I and *Bam*HI to extract a 1.5 Kb fragment that was subsequently subcloned into pAcC4 (*Bio/Technology* 6:47-55, 1988), a derivative of pAc436. Generation of the recombinant baculovirus was achieved by co-transfecting 2 μ g of the HIV p55 Gag pAcC4 shuttle vector with 0.5 μ g of linearized, *Autographa californica* baculovirus (AcNPV) wild-type viral DNA into *Spodoptera frugiperda* (Sf9) cells (Kitts, P.A., Ayres M.D., and Possee R.D., *Nucleic Acids Res.* 18:5667-5672, 1990). The isolation of recombinant virus expressing HIV p55 Gag was performed according to standard techniques (O'Reilly, D.R., L.K. Miller, and V. A. Luckow, *Baculovirus Expression Vector: A Laboratory Manual*, W.H. Freeman and Company, New York, 1992).

Expression of the HIV p55 Gag was achieved using a 500 ml suspension culture of Sf9 cells grown in serum-free medium (Miaorella, B., D. Inlow, A. Shauger, and D. Harano, *Bio/Technology* 6:1506-1510, 1988) that had been infected with the HIV p55 Gag recombinant baculovirus at a multiplicity of infection (MOI) of 10. Forty-eight hours post-infection, the supernatant was separated by centrifugation and filtered through a 0.2 μ m filter. Aliquots of the supernatant were then transferred to Polyclear™ (Beckman Instruments, Palo Alto, CA) ultracentrifuge tubes, underlaid with 20% (wt/wt) sucrose, and subjected to 2 hours centrifugation at 24,000 rpm using a Beckman SW28 rotor.

The resulting pellet was suspended in Tris buffer (20 mM Tris HCl, pH 7.5, 250 mM NaCl, and 2.5 mM ethylenediaminetetraacetic acid [EDTA]), layered onto a 20-60% (wt/wt) sucrose gradient, and subjected to 2 hours centrifugation at 40,000 rpm using a Beckman SW41ti

rotor. The gradient was then fractionated starting at the top (20% sucrose) of the gradient into approximately twelve 0.75 ml aliquots. A sample of each fraction was electrophoresed on 8-16% SDS polyacrylamide gels and the resulting bands were visualized after commassie staining (Figure 4). Additional aliquots were subjected to refractive index analysis.

The results shown in Figure 4 indicated that the p55 Gag virus-like particles banded at a sucrose density of range of 1.15 - 1.19 g/ml with the peak at approximately 1.17 g/ml. The peak fractions were pooled and concentrated by a second 20% sucrose pelleting. The resulting pellet was suspended in 1 ml of Tris buffer (described above). The total protein yield as estimated by Bicimchrominic Acid (BCA) (Pierce Chemical, Rockford, IL) was 1.6 mg.

B. Expression of Synthetic HIV p55 Gag

A baculovirus shuttle vector containing the synthetic p55 Gag sequence was constructed as follows. The synthetic HIV p55 expression cassette (Example 1) was digested with restriction enzyme *SalI* followed by incubation with T4-DNA polymerase. The resulting fragment was isolated (PCR Clean-Up™, Promega, Madison, WI) and then digested with *BamHI* endonuclease. The shuttle vector pAcC13 (Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990) was linearized by digestion with *EcoI*, followed by incubation with T4-DNA polymerase, and then isolated (PCR Clean-Up™). The linearized vector was digested with *BamHI*, treated with alkaline phosphatase, and isolated by size fragmentation in an agarose gel. The isolated 1.5 kb fragment was ligated with the prepared pAcC13 vector. The resulting clone was designated pAcC13-Modif.p55Gag.

The expression conditions for the synthetic HIV p55 VLPs differed from those of the native p55 Gag as follows: a culture volume of 1 liter used instead of 500 ml; *Trichoplusia ni* (Tn5) (Wickham, T.J., and Nermerow, G.R., *BioTechnology Progress*, 9:25-30, 1993) insect cells were used instead of Sf9 insect cells; and, an MOI of 3 was instead of an MOI of 10. Experiments performed in support of the present invention showed that there was no appreciable difference in expression level between the Sf9 and Tn5 insect cells with the native p55 clone. In terms of MOI, experience with the native p55 clone suggested that an MOI of 10 resulted in higher expression (approximately 2-fold) of VLPs than a lower MOI.

The sucrose pelleting and banding methods used for the synthetic p55 VLPs were similar to those employed for the native p55 VLPs (described above), with the following exceptions: pelleted VLPs were suspended in 4 ml of phosphate buffered saline (PBS) instead of 1.0 ml of the Tris buffer; and four, 20-60% sucrose gradients were used instead of a single gradient. Also, due to the high concentration of banded VLPs, further concentration by pelleting was not required. The peak fractions from all 4 gradients were simply dialyzed against PBS. The approximate density of the banded VLPs ranged from 1.23-1.28 g/ml. A total protein yield as estimated by BCA was 46 mg. Results from the sucrose gradient banding of the synthetic p55 are shown in Figure 5.

A comparison of the total amount of purified HIV p55 Gag from several preparations obtained from the two baculovirus expression cassettes has been summarized in Figure 6. The average yield from the native p55 was 3.16 mg/liter of culture (n=5, standard deviation (sd) ± 1.07 , range = 1.8-4.8 mg/L) whereas the average yield from the

synthetic p55 was more than ten-fold higher at 44.5 mg/liter of culture ($n=2$, $sd=\pm 6.4$).

In addition to a higher total protein yield, the final product from the synthetic p55-expressed Gag consistently contained lower amounts of contaminating baculovirus proteins than the final product from the native p55-expressed Gag. This difference can be seen in the two commassie-stained gels Figures 4 and 5.

10 C. Expression of Native and Synthetic Gag-Core

Expression of the HIV p55 Gag/HCV Core 173 (SEQ ID NO:8) was achieved using a 2.5 liter suspension culture of Sf9 cells grown in serum-free medium (Miaorella, B., D. Inlow, A. Shauger, and D. Harano. 1988 Bio/Technology 6:1506-1510). The cells were infected with an HIV p55 Gag/HCV Core 173 recombinant baculovirus. Forty-eight hours post-infection, the supernatant was separated from the cells by centrifugation and filtered through a 0.2 μ m filter. Aliquots of the supernatant were then transferred to a Polyclear™ (Beckman Instruments, Palo Alto, CA) ultracentrifuge tubes containing 30% (wt/wt) sucrose, and subjected to 2 hours of centrifugation at 24,000 rpm in a Beckman SW28 rotor and ultracentrifuge.

The resulting pellet was suspended in Tris buffer (50 mM Tris-HCl, pH 7.5, 500 mM NaCl) and layered onto a 30-60% (wt/wt) sucrose gradient and subjected to 2 hours centrifugation at 40,000 rpm in a Beckman SW41ti rotor and ultracentrifuge. The gradient was then fractionated starting at the top (30%) of the gradient into approximately 11 x 1.0 ml aliquots. A sample of each fraction was electrophoresed on 8-16% SDS polyacrylamide gels and the resulting bands were visualized after commassie staining.

A subset of aliquots were also subjected to Western blot analysis using monoclonal antibody 76C.5EG (Steimer, K.S., et al., Virology 150:283-290, 1986) which is specific for HIV p24 (a subunit of HIV p55). The peak fractions from the sucrose gradient were pooled and concentrated by a second 20% sucrose pelleting. The resulting pellet was suspended in 1 ml of buffer Tris buffer and the total protein yield as estimated by BCA (Pierce Chemical, Rockford, IL) was ~ 1.0 mg.

The results from the SDS PAGE are shown in Figure 8 and the anti- p24 Western blot results are shown in Figure 9. Taken together, these results indicate that the HIV p55 Gag/HCV Core 173 chimeric VLPs banded at a sucrose density similar to that of the HIV p55 Gag VLPs and the visible protein band that migrated at a molecular weight of ~ 72,000 kd was reactive with the HIV p24-specific monoclonal antibody. An additional immunoreactive band at approximately 55,000 kd also appeared to be reactive with the anti-p24 antibody and may be a degradation product.

Although aliquots from the above preparation were not tested for reactivity with an HCV Core-specific antibody (an anti-CD22 rabbit serum), results from a similar preparation are shown in Figure 10 and indicate that the main HCV Core-specific reactivity migrates at an approximate molecular weight of 72,000 kd which is in accordance with the predicted molecular weight of the chimeric protein.

The expression conditions for the synthetic HIV p55 Gag/HCV Core 173 (SEQ ID NO:8) VLPs differed from those of the native p55 Gag and are as follows: a culture volume of 1 liter used instead of 2.5 liters, *Trichoplusia ni* (Tn5) (Wickham, T.J., and Nemerow, G.R. 1993 BioTechnology Progress, 9:25-30) insect cells were

used instead of Sf9 insect cells and an MOI of 3 was instead of an MOI of 10. The sucrose pelleting and banding methods used for the synthetic HIV p55 Gag/HCV Core 173 VLPs were similar to those employed for the native HIV p55 Gag/HCV Core 173 VLPs. However, differences included: pelleted VLPs were suspended in 1 ml of phosphate buffered saline (PBS) instead of 1.0 ml of the Tris buffer, and a single 20-60% sucrose gradients was used. A comparison of the total amount of purified HIV p55 Gag/HCV Core 173 from multiple preparations obtained from the two baculovirus expression cassettes showed that there was an increase in expression using the synthetic HIV p55 Gag/HCV Core 173 cassette.

D. Alternative method for the enrichment of HIV p55 Gag VLPs

In addition to purification from the media, p55 (Gag protein) expressed in baculovirus (e.g., using a synthetic expression cassette of the present invention) can also be purified as virus-like particles from the infected insect cells. For example, forty-eight hours post infection, the media and cell pellet are separated by centrifugation and the cell pellet is stored at -70°C until future use. At the time of processing, the cell pellet is suspended in 5 volumes of hypotonic lysis buffer (20 mM Tris-HCl, pH 8.2, 1 mM EGTA; 1 mM MgCl₂, and Complete Protease Inhibitor® (Boehringer Mannheim Corp., Indianapolis, IN)). If needed, the cells are then dounced 8-10 times to complete cell lysis.

The lysate is then centrifuged at approximately 1000-1500 x g for 20 minutes. The supernatant is

decanted into UltraClear™ tubes, underlayered with 20% sucrose (w/w) and centrifuged at 24,000 rpm in SW28 buckets for 2 hours. The resulting pellet is suspended in Tris buffer (20 mM Tris HCl, pH 7.5, 250 mM NaCl, and 2.5 mM ethylenediamine-tetraacetic acid (EDTA) with 0.1% IGEPAL detergent (Sigma Chemical, St. Louis, MO) and 250 units/ml of benzonase (American International Chemical, Inc., Natick, MA) and incubated at 4°C for at least 30 minutes. The suspension is subsequently layered onto a 20-60% sucrose gradient and spun at 40,000 rpm using an SW41ti rotor for 20-24 hours.

After ultracentrifugation, the sucrose gradient is fractionated and aliquots run on SDS PAGE to identify peak fractions. The peak fractions are dialyzed against PBS and measured for protein content. Negatively stained electron micrographs typically show non-enveloped VLPs somewhat smaller in diameter (80-120 nm) than the budded VLPs. HIV Gag VLPs prepared in this manner are also capable of generating Gag-specific CTL responses in mice.

Example 8

In Vivo Immunogenicity of Synthetic Gag Expression Cassettes

A. Immunization

To evaluate the possibly improved immunogenicity of the synthetic Gag expression cassettes, a mouse study was performed. The plasmid DNA, pCMVKM2 carrying the synthetic Gag expression cassette, was diluted to the following final concentrations in a total injection volume of 100 µl: 20 µg, 2 µg, 0.2 µg, and 0.02 µg. To

overcome possible negative dilution effects of the diluted DNA, the total DNA concentration in each sample was brought up to 20 μ g using the vector (pCMVKM2) alone. As a control, plasmid DNA of the native Gag expression cassette was handled in the same manner. Twelve groups of four Balb/c mice (Charles River, Boston, MA) were intramuscularly immunized (50 μ l per leg, intramuscular injection into the *tibialis anterior*) according to the schedule in Table 7.

Table 7

Group	Gag Expression Cassette	Concentration of Gag plasmid DNA (μ g)	Immunized at time (weeks):
1	Synthetic	20	0 ¹ , 4
2	Synthetic	2	0, 4
3	Synthetic	0.2	0, 4
4	Synthetic	0.02	0, 4
5	Synthetic	20	0
6	Synthetic	2	0
7	Synthetic	0.2	0
8	Synthetic	0.02	0
9	Native	20	0
10	Native	2	0
11	Native	0.2	0
12	Native	0.02	0

1 = initial immunization at "week 0"

Groups 1-4 were bled at week 0 (before immunization), week 4, week 6, week 8, and week 12. Groups 5-12 were bled at week 0 (before immunization) and at week 4.

B. Humoral Immune Response

The humoral immune response was checked with an anti-HIV Gag antibody ELISAs (enzyme-linked immunosorbent assays) of the mice sera 0 and 4 weeks post immunization (groups 5-12) and, in addition, 6 and 8 weeks post immunization, respectively, 2 and 4 weeks post second immunization (groups 1-4).

The antibody titers of the sera were determined by anti-Gag antibody ELISA. Briefly, sera from immunized mice were screened for antibodies directed against the HIV p55 Gag protein. ELISA microtiter plates were coated with 0.2 μ g of HIV-1_{SF2} p24-Gag protein per well overnight and washed four times; subsequently, blocking was done with PBS-0.2% Tween (Sigma) for 2 hours. After removal of the blocking solution, 100 μ l of diluted mouse serum was added. Sera were tested at 1/25 dilutions and by serial 3-fold dilutions, thereafter. Microtiter plates were washed four times and incubated with a secondary, peroxidase-coupled anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA plates were washed and 100 μ l of 3, 3', 5, 5'-tetramethyl benzidine (TMB; Pierce) was added per well. The optical density of each well was measured after 15 minutes. The titers reported are the reciprocal of the dilution of serum that gave a half-maximum optical density (O.D.). The ELISA results are presented in Table 8.

Table 8

Group	Inoculum (μ g)	Expression cassette	Sera - Week 4 ³	Sera - Week 6	Sera - Week 8
1	20	S ¹ - gag	98	455	551
2	2	S - gag	59	1408	227
3	0.	S - gag	29	186	61
4	0.02	S - gag	< 20	< 20	< 20
5	20	S - gag	67	n.a. ⁴	n.a.
6	2	S - gag	63	n.a.	n.a.
7	0.	S - gag	57	n.a.	n.a.
8	0.02	S - gag	< 20	n.a.	n.a.
9	20	N ² - gag	43	n.a.	n.a.
10	2	N - gag	< 20	n.a.	n.a.
11	0.	N - gag	< 20	n.a.	n.a.
12	0.02	N - gag	< 20	n.a.	n.a.

1 = synthetic gag expression cassette (SEQ ID NO: 4)

2 = native gag expression cassette (SEQ ID NO: 1)

3 = geometric mean antibody titer

4 = not applicable

The results of the mouse immunizations with plasmid-DNAs show that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression cassettes. Also, the second boost immunization induced a secondary immune response after two weeks (groups 1-3).

C. Cellular Immune Response

The frequency of specific cytotoxic T-lymphocytes (CTL) was evaluated by a standard chromium release assay of peptide pulsed Balb/c mouse CD4 cells. Gag expressing vaccinia virus infected CD-8 cells were used as a positive control (vvGag). Briefly, spleen cells (Effector cells, E) were obtained from the BALB/c mice immunized as described above (Table 8) were cultured, restimulated, and assayed for CTL activity against Gag

peptide-pulsed target cells as described (Doe, B., and Walker, C.M., *AIDS* 10(7):793-794, 1996). The HIV-1_{SF2} Gag peptide used was p7g SEQ ID NO:10. Cytotoxic activity was measured in a standard ⁵¹Cr release assay. Target (T) cells were cultured with effector (E) cells at various E:T ratios for 4 hours and the average cpm from duplicate wells was used to calculate percent specific ⁵¹Cr release. The results are presented in Table 9.

Cytotoxic T-cell (CTL) activity was measured in splenocytes recovered from the mice immunized with HIV Gag DNA (compare Effector column, Table 9, to immunization schedule, Table 8). Effector cells from the Gag DNA-immunized animals exhibited specific lysis of Gag p7g peptide-pulsed SV-BALB (MHC matched) targets cells indicative of a CTL response. Target cells that were peptide-pulsed and derived from an MHC-unmatched mouse strain (MC57) were not lysed (Table 9; MC/p7g).

Table 9

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10

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Immunization	E:T	Percent specific lysis of target cells		
		SVBALB none	SVBALB p7g	RMA p7g
20 µg DNA gagmod	100:1	2	49	<1
	30:1	3	30	<1
	10:1	<1	14	<1
2 µg DNA gagmod	100:1	2	37	<1
	30:1	2	21	<1
	10:1	<1	13	<1
0.2 µg DNA gagmod	100:1	2	32	<1
	30:1	3	25	<1
	10:1	1	14	<1
0.02 µg DNA gagmod	100:1	1	17	<1
	30:1	1	16	<1
	10:1	1	8	<1
20 µg DNA gag native	100:1	2	49	<1
	30:1	2	24	<1
	10:1	1	12	<1
2 µg DNA gag native	100:1	<1	18	<1
	30:1	1	14	<1
	10:1	1	7	<1
0.2 µg DNA gag native	100:1	3	30	<1
	30:1	3	17	<1
	10:1	2	7	<1
0.02 µg DNA gag native	100:1	4	2	<1
	30:1	1	2	<1
	10:1	1	2	<1

representative results of two animals per DNA-dose; positive CTL responses are indicated by boxed data

The results of the CTL assays show increased potency of synthetic Gag expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

Example 9In vivo Immunization with Env polypeptidesA. Immunogenicity Study of US4 o-gp140 in Ras-3c Adjuvant System

5 Studies have been conducted using rabbits immunized with US4 o-gp140 purified as described above. Studies are also underway in animals to determine immunogenicity of US4 gp120, SF162 o-gp140 and SF162 gp120.

10 Two rabbits (#1 and #2) were immunized intramuscularly at 0, 4, 12 and 24 weeks with 50 µg of US4 o-gp140 in the RibitTM adjuvant system (RAS-3c), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL, Ribi Immunochem, Hamilton, MT).

15 In each experiment described herein, o-gp140 can be native, mutated and/or modified. Antibody responses directed against the US4 o-gp140 protein were measured by ELISA. Results are shown in Table 10.

Table 10

Rabbit/sample	Approximate o-gp140 ELISA titer
pre-immunization	0
#1: post1 (0 week immuniz)	400
#1: post2 (4 week immuniz)	15,000
#1: post3 (12 week immuniz)	50,000
#1: post4 (24 week immuniz)	100,000
#2: post1 (0 week immuniz)	600
#2: post2 (4 week immuniz)	12,000
#2: post3 (12 week immuniz)	25,000
#2: post4 (24 week immuniz)	55,000

The avidities of antibodies directed against the US4 o-gp140 protein were measured in a similar ELISA format employing successive washes with increasing concentrations of ammonium isothiocyanate. Results are shown in Table 11.

Table 11

Time of sample	Approx. Antibody avidity (NH ₄ HCN Conc. in M)
pre-immunization	0.02
post1 (0 week immuniz)	1.8
post2 (4 week immuniz)	3.5
post3 (12 week immuniz)	5.5
post4 (24 week immuniz)	5.1

These results show that US4 o-gp140 is highly immunogenic and able to induce substantial antibody responses after only one or two immunizations.

5 B. Immunogenicity of US4 o-gp140 in MF59-based Adjuvants

Groups of 4 rabbits were immunized intramuscularly at 0, 4, 12 and 24 weeks with various doses of US4 o-gp140 protein in three different MF59-based adjuvants (MF59 is described in International Publication No. WO 90/14837 and typically contains 5% Squalene, 0.5% Tween 80, and 0.5% Span 85). Antibody titers were measured post-third by ELISA using SF2 gp120 to coat the plates. QHC is a quill-based adjuvant (Iscotek, Uppsala, Sweden). Results are shown in Table 12.

15 Table 12

Antigen dose (μ g)	Adjuvant	Anti-gp120 _{SF2} Ab GMT*
12.5	MF59	7231
25	MF59	8896
50	MF59	12822
12.5	MF59/MPL	24146
25	MF59/MPL	27199
50	MF59/MPL	23059
50	MF59/MPL/QHC	31759

*GMT = geometric mean titer

Thus, adjuvanted o-gp140 generated antigen-specific antibodies. Further, the antibodies were shown to increased in avidity over time.

30 C. Neutralizing Antibodies

Neutralizing antibodies post-third immunization were measured against HIV-1 SF2 in a T-cell line adapted virus

(TCLA) assay and against PBMC-grown HIV-1 variants SF2, SF162 and 119 using the CCR5+ CEMx174 LTR-GFP reporter cell line, 5.25 (provided by N. Landau, Salk Institute, San Diego, CA) as target cells. Results are shown in Table 13.

Table 13

Neutralizing antibody responses in rabbits immunized with o-gp140.modUS4 protein

Group	Animal	SF2 TCLA*	SF2 PBMC [#]	SF162 PBMC [#]	119 PBMC [#]
Experiment 1					
o-gp140/ Ras-3c 50 mg	217	>640	100%	49	17
	218	>640	96	37	29
Experiment 2					
o-gp140/ MF59 50 mg	792	45	71	39	26
	793	50	87	26	4
	794	59	87	13	0
	795	128	92	15	0
o-gp140/ MF59 + MPL 50 mg	804	173	91	47	18
	805	134	93	28	4
	806	N.D.**	95	49	13
	807	441	100	31	15
o-gp140/MF59 + MPL + QHC 50 mg	808	465	98	46	40
	809	496	100	44	39
	810	>640	101	27	4
	811	92	92	24	37

*TCLA neutralizing antibody titers (50% inhibition).

**Not Determined

[#] % Inhibition at 1:10 dilution of sera with any detectable non-specific inhibition in pre-bleeds subtracted.

The above studies in rabbits indicate that the US4 o-gp140 protein is highly immunogenic. When administered with adjuvant, this protein was able to induce substantial antibody responses after only one or two immunizations. Moreover, the adjuvanted o-gp140 protein was able to generate antigen-specific antibodies which increased in avidity after successive immunizations, and substantial neutralizing activity against T-cell line adapted HIV-1. Neutralizing activity was also observed against PBMC-grown primary HIV strains, including the difficult to neutralize CCR5 co-receptor (R5)-utilizing isolates, SF162 and 119.

Example 10

In Vivo Immunogenicity of Synthetic Env Expression

Cassettes

A. General Immunization Methods

To evaluate the immunogenicity of the synthetic Env expression cassettes, studies using guinea pigs, rabbits, mice, rhesus macaques and baboons were performed. The studies were structured as follows: DNA immunization alone (single or multiple); DNA immunization followed by protein immunization (boost); DNA immunization followed by Sindbis particle immunization; immunization by Sindbis particles alone.

B. Humoral Immune Response

The humoral immune response was checked in serum specimens from immunized animals with an anti-HIV Env antibody ELISAs (enzyme-linked immunosorbent assays) at various times post-immunization. The antibody titers of the sera were determined by anti-Env antibody ELISA as described above. Briefly, sera from immunized animals were

screened for antibodies directed against the HIV gp120 or gp140 Env protein. Wells of ELISA microtiter plates were coated

overnight with the selected Env protein and washed four
5 times; subsequently, blocking was done with PBS-0.2% Tween
(Sigma) for 2 hours. After removal of the blocking
solution, 100 μ l of diluted mouse serum was added. Sera
were tested at 1/25 dilutions and by serial 3-fold
dilutions, thereafter. Microtiter plates were washed four
10 times and incubated with a secondary, peroxidase-coupled
anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA
plates were washed and 100 μ l of 3, 3', 5, 5'-tetramethyl
benzidine (TMB; Pierce) was added per well. The optical
density of each well was measured after 15 minutes. Titers
15 are typically reported as the reciprocal of the dilution of
serum that gave a half-maximum optical density (O.D.).

Example 11

DNA-immunization of Baboons Using Synthetic Gag 20 Expression Cassettes

A. Baboons

Four baboons were immunized 3 times (weeks 0, 4 and 8)
bilaterally, intramuscular into the quadriceps using 1mg
pCMVKM2.GagMod.SF2 plasmid-DNA (Example 1). The animals
25 were bled two weeks after each immunization and a p24
antibody ELISA was performed with isolated plasma. The
ELISA was performed essentially as described in Example 5
except the second antibody-conjugate was an anti-human IgG,
g-chain specific, peroxidase conjugate (Sigma Chemical Co.,
30 St. Louis, MD 63178) used at a dilution of 1:500. Fifty
 μ g/ml yeast extract was added to the dilutions of plasma

samples and antibody conjugate to reduce non-specific background due to

preexisting yeast antibodies in the baboons. The antibody titer results are presented in Table 14.

5

Table 14

Immunization no.	Weeks	Antigen	wpi ^a / Baboon No.	Ab-titer ^b
10	0	gagmod DNA	0 w/219	< 10
			0 w/220	< 10
			0 w/221	< 10
			0 w/222	< 10
15	6		2 wp 1st/219	< 10
			2 wp 1st/220	< 10
			2 wp 1st/221	< 10
			2 wp 1st/222	15
20	14	gagmod DNA	2 wp 4th/219	< 10
			2 wp 4th/220	88
			2 wp 4th/221	< 10
			2 wp 4th/222	56
25	30	gagmod DNA	2 wp 5th/219	< 10
			2 wp 5th/220	391
			2 wp 5th/221	237
			2 wp 5th/222	222
30	46	gag VLP protein	2 wp 6th/219	753
			2 wp 6th/219	4330
			2 wp 6th/219	5000
			2 wp 6th/219	2881

^a wpi = weeks post immunization

^b geometric mean antibody titer

30

In Table 14, pre-bleed data are given as Immunization No. 0; data for bleeds taken 2 weeks post-first immunization are given as Immunization No. 1; data for bleeds taken 2 weeks post-second immunization are given as Immunization No. 2; and, data for bleeds taken 2 weeks post-third immunization are given as Immunization No. 3.

35

Further, lymphoproliferative responses to p24 antigen were also observed in baboons 221 and 222 two weeks post-fourth immunization (at week 14), and enhanced substantially post-boosting with VLP (at week 44 and 76).
5 Such proliferation results are indicative of induction of T-helper cell functions.

B. Rhesus Macaques

The improved potency of the codon-modified gag expression plasmid observed in mouse and baboon studies was confirmed in rhesus macaques. Four of four macaques had detectable Gag-specific CTL after two or three 1 mg doses of modified gag plasmid. In contrast, in a previous study, only one of four macaques given 1 mg
10 doses of plasmid-DNA encoding the wild-type HIV-1_{sf2} Gag showed strong CTL activity that was not apparent until after the seventh immunization. Further evidence of the potency of the modified gag plasmid was the observation that CTL from two of the four rhesus macaques reacted
15 with three nonoverlapping Gag peptide pools, suggesting that as many as three different Gag peptides are recognized and indicating that the CTL response is polyclonal. Additional quantification and specificity studies are in progress to further characterize the T
20 cell responses to Gag in the plasmid-immunized rhesus macaques. DNA immunization of macaques with the modified gag plasmid did not result in significant antibody responses, with only two of four animals seroconverting at low titers. In contrast, in the same study the
25 majority of macaques in groups immunized with p55Gag protein seroconverted and had strong Gag-specific antibody titers. These data suggest that a prime-boost
30

strategy (DNA-prime and protein-boost) could be very promising for the induction of a strong CTL and antibody response.

5 In sum, these results demonstrate that the synthetic Gag plasmid DNA is immunogenic in non-human primates. When similar experiments were carried out using wild-type Gag plasmid DNA no such induction of anti-p24 antibodies was observed after four immunizations.

10

Example 12

DNA- and Protein Immunizations of Animals Using Env Expression Cassettes and Polypeptides

A. Guinea Pigs

Groups comprising six guinea pigs each were
15 immunized intramuscularly at 0, 4, and 12 weeks with plasmid DNAs encoding the gp120.modUS4, gp140.modUS4, gp140.modUS4.delV1, gp140.modUS4.delV2, gp140.modUS4.delV1/V2, or gp160.modUS4 coding sequences of the US4-derived Env. The animals were subsequently
20 boosted at 18 weeks with a single intramuscular dose of US4 o-gp140.mut.modUS4 protein in MF59 adjuvant. Anti-gp120 SF2 antibody titers (geometric mean titers) were measured at two weeks following the third DNA
immunization and ~~at~~ two weeks after the protein boost.
25 Results are shown in Table 15.

Table 15

Group	GMT post-DNA immuniz.	GMT post-protein boost
gp120.modUS4	2098	9489
gp140.modUS4	190	5340
gp140.modUS4.delV1	341	7808
gp140.modUS4.delV2	386	8165
gp140.modUS4.delV1/V 2	664	8270
gp160.modUS4	235	9928

These results demonstrate the usefulness of the synthetic constructs to generate immune responses, as well as, the advantage of providing a protein boost to enhance the immune response following DNA immunization.

B. Rabbits

Rabbits were immunized intramuscularly and intradermally using a Bioject needless syringe with plasmid DNAs encoding the following synthetic SF162 Env polypeptides: gp120.modSF162, gp120.modSF162.delV2, gp140.modSF162, gp140.modSF162.delV2, gp140.mut.modSF162, gp140.mut.modSF162.delV2, gp160.modSF162, and gp160.modSF162.delV2. Approximately 1 mg of plasmid DNA (pCMVlink) carrying the synthetic Env expression cassette was used to immunize the rabbits. Rabbits were immunized with plasmid DNA at 0, 4, and 12 weeks. At two weeks after the third immunization all of the constructs were shown to have generated significant antibody titers in the test animals. Further, rabbits immunized with constructs containing deletions of the V2 region

generally generated similar antibody titers relative to rabbits immunized with the companion construct still containing the V2 region.

The nucleic acid immunizations are followed by protein boosting with o-gp140.modSF162.delV2 (0.1 mg of purified protein) at 24 weeks after the initial immunization. Results are shown in Table 16.

Table 16

Group	GMT 2wks post-2nd DNA immunization	GMT 2wks post-3rd DNA immunization	GMT 2wks post-protein boost
gp120.modSF162	4573	5899	26033
gp120.modSF162.delV2	3811	3122	29606
gp140.modSF162	1478	710	12882
gp140.modSF162.delV2	1572	819	11067
gp140.mut.modSF162	1417	788	8827
gp140.mut.modSF162.delV2	1378	1207	13301
gp160.modSF162	23	81	7050
gp160.modSF162.delV2	85	459	11568

All constructs are highly immunogenic and generate substantial antigen binding antibody responses after only 2 immunizations in rabbits.

C. Baboons

Groups of four baboons were immunized intramuscularly with 1 mg doses of DNA encoding different forms of synthetic US4 gp140 (see the following table) at 0, 4, 8, 12, 28, and 44 weeks. The animals were also boosted twice with US4 0-gp140 protein (gp140.mut.modUS4) at 44 and 76 weeks using MF59 as adjuvant. Results are shown in Table 17.

Table 17				
Animal	Treatment	2 Wks Post 5th DNA immuniza- tion	2 Wks post 6th DNA (plus o- gp140 prot. immuniz.)	2 Wks post 7th DNA (o-gp140 protein only)
CY 215	gp140.modUS4	8.3	446	1813
CY 216		8.3	433	1236
CY 217		68	1660	2989
CY 218		101	2556	1610
Geomean:		26.2	951.4	1812.1
CY 219	gp140.modUS4 + p55gag.SF2	8.3	8.3	421
CY 220		8.3	8.3	3117
CY 221		8.3	954	871
CY 222		8.3	71	916
Geomean:		8.3	46.5	1011.5
CY 223	gp140.mut. modUS4	41.4	10497	46432
CY 224		8.3	979	470
CY 225		135	2935	3870
CY 226		47	1209	4009
Geomean:		68.3	2457.4	4289.6
CY 227	gp140TM. modUS4	8.3	56	5001
CY 228		8.3	806	1170
CY 229		8.3	48	3402
CY 230		8.3	38	6520
GMT*:		8.3	95.3	3375.3

*GMT = geometric mean titer

The results in Table 17 demonstrate the usefulness of the synthetic constructs to generate immune responses in primates such as baboons. In addition, all animals

showed evidence of antigen-specific (*Env* antigen) lymphoproliferative responses.

D. Rhesus Macaques

5 Two rhesus macaques (designated H445 and J408) were immunized with 1 mg of DNA encoding SF162 gp140 with a deleted V2 region (SF162.gp140.delV2) by intramuscular (IM) and intradermal (ID) routes at 0, 4, 8, and 28 weeks. Approximately 100 μ g of the protein encoded by
10 the SF162. gp140mut.delV2 construct was also administered in MF59 by IM delivery at 28 weeks.

ELISA titers are shown in Figure 61. Neutralizing antibody activity is shown Tables 18 and 19. Neutralizing antibody activity was determined against a
15 variety of primary HIV-1 isolates in a primary lymphocyte or "PBMC-based" assay (see the following tables). Further, the phenotypic co-receptor usage for each of the primary isolates is indicated. As can be seen in the
tables neutralizing antibodies were detected against
20 every isolate tested, including the HIV-1 primary isolates (i.e., SF128A, 92US660, 92HT593, 92US657, 92US714, 91US056, and 91US054).

Table 18					
	Treatment		Bleed 0	Bleed 1	Bleed 2
Animal	1st Immunization	2nd Immunization	1st Imm'n	2nd Imm'n	2 Wks post 2nd
5 EO 456	25µg 120mod DNA	(None)	8.3	45	309
EO 457			8.3	254	460
EO 458			8.3	8.3	93
EO 459			8.3	43	45
EO 460			8.3	8.3	274
10 EO 461	25µg 120mod DNA	25µg 120mod DNA	8.3	47	1502
EO 462			8.3	80	5776
EO 463			8.3	89	3440
EO 464			8.3	8.3	3347
EO 465			8.3	69	1127
15 EO 466	50µg 120mod DNA	(None)	8.3	63	102
EO 467			8.3	112	662
EO 468			8.3	94	459
EO 469			8.3	58	48
EO 470			8.3	95	355
20 EO 471	50µg 120mod DNA	50µg 120mod DNA	8.3	110	9074
EO 472			8.3	8.3	4897
EO 473			8.3	49	4089
EO 474			8.3	59	5280
EO 475			8.3	8.3	929
25 EO 476	25µg 120mod DNA	Sindbis/Env	8.3		653
EO 477			8.3	87	22675
EO 478			8.3	76	3869
EO 479			8.3		1004
EO 480			8.3	71	7080

Table 19					
	Treatment		Bleed 0	Bleed 1	Bleed 2
Animal	1st Immunization	2nd Immunization	1st Imm'n	2nd Imm'n	2 Wks post 2nd
EO 481	Sindbis/Env	(None)	8.3	8.3	8.3
EO 482			8.3	8.3	8.3
EO 483			8.3	78	103
EO 484			8.3	8.3	32
EO 485			8.3	76	207
EO 486	Sindbis/Env	Sindbis/Env	8.3	8.3	458
EO 487			8.3	8.3	345
EO 488			8.3	8.3	331
EO 489			8.3	103	111
EO 490			8.3	8.3	5636

Lymphoproliferative activity (LPA) was also determined by antigenic stimulation followed by uptake of ³H-thymidine in these animals and is shown in Table 20. Experiment 1 was performed at 14 weeks post third DNA immunization and Experiment 2 was performed at 2 weeks post fourth DNA immunization using DNA and protein. For gp120ThaiE, gp120SF2 and US4 o-gp140, appropriate background values were used to calculate Stimulation Indices (S.I.; Antigenic stimulation CPM/Background CPM).

Table 20

S.I.: Calculated as Ag CPM/Background CPM				
Animal/ exp#	gp120Thai E	gp120 SF2	env2-3SF2	o- gp140US4
J408/#1	2	1	1	5
H445/#1	1	1	1	6
J408/#2	1	1	2	3
H445/#2	0	0	3	2

As can be seen by the results presented in Table 20 lymphoproliferative responses to o-gp140.US4 antigen were also in all four animals at both experimental time points. Such proliferation results are indicative of induction of T-helper cell functions.

The results presented above demonstrate that the synthetic gp140.modSF162.delV2 DNA and protein are immunogenic in non-human primates.

Example 13

In vitro expression of recombinant Sindbis RNA and DNA containing the synthetic Gag or Env expression cassettes

5 A. Synthetic Gag expression cassettes

To evaluate the expression efficiency of the synthetic Gag expression cassette in Alphavirus vectors, the synthetic Gag expression cassette was subcloned into both plasmid DNA-based and recombinant vector particle-based Sindbis virus vectors. Specifically, a cDNA vector construct for in vitro transcription of Sindbis virus RNA vector replicons (pRSIN-luc; Dubensky, et al., *J Virol.* 70:508-519, 1996) was modified to contain a *PmeI* site for plasmid linearization and a polylinker for insertion of heterologous genes. A polylinker was generated using two oligonucleotides that contain the sites *XhoI*, *PmlI*, *ApaI*, *NarI*, *XbaI*, and *NotI* (XPANXNF, SEQ ID NO:17, and XPANXNR, SEQ ID NO:18).

The plasmid pRSIN-luc (Dubensky et al., supra) was digested with *XhoI* and *NotI* to remove the luciferase gene insert, blunt-ended using Klenow and dNTPs, and purified from an agarose gel using GeneCleanII (Biol01, Vista, CA). The oligonucleotides were annealed to each other and ligated into the plasmid. The resulting construct was digested with *NotI* and *SacI* to remove the minimal Sindbis 3'-end sequence and A_{40} tract, and ligated with an approximately 0.4 kbp fragment from PKSSIN1-BV (WO 97/38087). This 0.4 kbp fragment was obtained by digestion of pKSSIN1-BV with *NotI* and *SacI*, and purification after size fractionation from an agarose gel. The fragment contained the complete Sindbis virus 3'-end, an A_{40} tract and a *PmeI* site for linearization. This new vector construct was designated SINBVE.

The synthetic HIV Gag coding sequence was obtained from the parental plasmid by digestion with *EcoRI*, blunt-ending with Klenow and dNTPs, purification with GeneCleanII, digestion with *Sall*, size fractionation on an agarose gel, and purification from the agarose gel using GeneCleanII. The synthetic Gag coding fragment was ligated into the SINBVE vector that had been digested with *XhoI* and *PmlI*. The resulting vector was purified using GeneCleanII and designated SINBVGag. Vector RNA replicons may be transcribed *in vitro* (Dubensky et al., *supra*) from SINBVGag and used directly for transfection of cells. Alternatively, the replicons may be packaged into recombinant vector particles by co-transfection with defective helper RNAs or using an alphavirus packaging cell line as described, for example, in U.S. Patent Numbers 5,843,723 and 5,789,245, and then administered *in vivo* as described..

The DNA-based Sindbis virus vector pDCMVSIN-beta-gal (Dubensky, et al., *J Virol.* 70:508-519, 1996) was digested with *Sall* and *XbaI*, to remove the beta-galactosidase gene insert, and purified using GeneCleanII after agarose gel size fractionation. The HIV Gag gene was inserted into the the pDCMVSIN-beta-gal by digestion of SINBVGag with *Sall* and *XhoI*, purification using GeneCleanII of the Gag-containing fragment after agarose gel size fractionation, and ligation. The resulting construct was designated pDSIN-Gag, and may be used directly for *in vivo* administration or formulated using any of the methods described herein.

BHK and 293 cells were transfected with recombinant Sindbis vector RNA and DNA, respectively. The supernatants and cell lysates were tested with the Coulter p24 capture ELISA (Example 2).

BHK cells were transfected by electroporation with recombinant Sindbis RNA. The expression of p24 (in ng/ml) is presented in Table 21. In the table, SINGag#1 and 2 represent duplicate measurements, and SIN β gal represents a negative control. Supernatants and lysates were collected 24h post transfection.

Table 21

Construct	Supernatant	Lysate
SIN β gal RNA	0	0
SINGag#1 RNA	7 ng	Max (approx. 1 μ g)
SINGag#2 RNA	1 ng	700 ng

293 cells were transfected using LT-1 (Example 2) with recombinant Sindbis DNA. Synthetic pCMVKM2GagMod.SF2 was used as a positive control. Supernatants and lysates were collected 48h post transfection. The expression of p24 (in ng/ml) is presented in Table 22.

Table 22

Construct	Supernatant	Lysate
SINGag DNA	3	30
pCMVKM2.GagMod.SF2 DNA	32	42

The results presented in Tables 21 and 22 demonstrate that Gag proteins can be efficiently expressed from both DNA and RNA-based Sindbis vector systems using the synthetic Gag expression cassette (p55Gag.mod).

B. Synthetic Env expression cassettes

To evaluate the expression efficiency of the synthetic Env expression cassette in Alphavirus vectors,

synthetic Env expression cassettes were subcloned into both plasmid DNA-based and recombinant vector particle-based Sindbis virus vectors as described above for Gag.

5 The synthetic HIV Env coding sequence was obtained from the parental plasmid by digestion with *SalI* and *XbaI*, size fractionation on an agarose gel, and purification from the agarose gel using GeneCleanII. The synthetic Env coding fragment was ligated into the SINBVE vector that had been digested with *XhoI* and *XbaI*. The
10 resulting vector was purified using GeneCleanII and designated SINBVEEnv. Vector RNA replicons may be transcribed *in vitro* (Dubensky et al., *supra*) from SINBVEEnv and used directly for transfection of cells. Alternatively, the replicons may be packaged into
15 recombinant vector particles by co-transfection with defective helper RNAs or using an alphavirus packaging cell line and administered as described above for Gag.

The DNA-based Sindbis virus vector pDCMVSIN-beta-gal (Dubensky, et al., *J Virol.* 70:508-519, 1996) was
20 digested with *SalI* and *XbaI*, to remove the beta-galactosidase gene insert, and purified using GeneCleanII after agarose gel size fractionation. The HIV Env gene was inserted into the the pDCMVSIN-beta-gal by digestion of SINBVEEnv with *XbaI* and *XhoI*, purification using
25 GeneCleanII of the Env-containing fragment after agarose gel size fractionation, and ligation. The resulting construct was designated pDSIN-Env, and may be used directly for *in vivo* administration or formulated using any of the methods described herein.

30 BHK and 293 cells were transfected with recombinant Sindbis vector RNA and DNA, respectively. The supernatants and cell lysates were tested by capture ELISA.

BHK cells were transfected by electroporation with recombinant Sindbis RNA. The expression of Env (in ng/ml) is presented in Table 23. In the table, the Sindbis RNA containing synthetic Env expression cassettes are indicated and β gal represents a negative control. Supernatants and lysates were collected 24h post transfection.

Table 23

Construct	Supernatant (Neat)ng/ml	Lysate (1:10 dilution)ng/ml
β gal RNA	0	0
gp140.modUS4	726	7147
gp140.modSF162	3529	7772
gp140.modUS4.delV1/V2	1738	6526
gp140.modUS4.delV2	960	3023
gp140.modSF162.delV2	2772	3359

293 cells were transfected using LT-1 mediated transfection (PanVera) with recombinant Sindbis DNA containing synthetic expression cassettes of the present invention and β gal sequences as a negative control. Supernatants and lysates were collected 48h post transfection. The expression of Env (in ng/ml) is presented in Table 24.

Table 24

Construct	Supernatant (Neat) ng/ml	Lysate (1:10 dilution) ng/ml
β gal	0	0
gp140.modSF162.delV2	1977	801
gp140.modSF162	949	746

The results presented in Tables 23 and 24 demonstrated that Env proteins can be efficiently expressed from both DNA and RNA-based Sindbis vector systems using the synthetic Env expression cassettes of the present invention.

Example 14

A. In vivo Immunization with Gag-containing DNA and/or Sindbis particles

CB6F1 mice were immunized intramuscularly at 0 and 4 weeks with plasmid DNA and/or Sindbis vector RNA-containing particles each containing GagMod.SF2 sequences as indicated in Table 25. Animals were challenged with recombinant vaccinia expressing SF2 Gag at 3 weeks post second immunization (at week 7). Spleens were removed from the immunized and challenged animals 5 days later for a standard ^{51}C release assay for CTL activity. Values shown in Table 25 indicate the results from the spleens of three mice from each group. The boxed values in Table 25 indicate that all groups of mice receiving immunizations with pCMVKm2.GagMod.SF2 DNA and/or SindbisGagMod.SF2 virus particles either alone or in combinations showed antigen-specific CTL activity.

Table 25

Cytotoxic T-lymphocyte (CTL) responses in mice immunized with HIV-1 gagmod DNA and Sindbis gagmod virus particles

5	Immunization	E:T	Percent specific lysis of target cells*		
			SVBALB	SVBALB	RMA
			none	p7g	p7g
10	pCMVKm2.GagMod.SF2 DNA ^a	100:1	5	20	1
	at 0, 4 wks	25:1	5	20	<1
		6:1	4	8	<1
10	SindbisGagMod.SF2 virus particles ^b	100:1	10	49	<1
	at 0, 4 weeks	25:1	7	20	<1
		6:1	5	12	<1
15	pCMVKm2.GagMod.SF2 DNA at 0 wks	100:1	9	58	<1
	SindbisGagMod.SF2 virus particles at 4 wks	25:1	7	42	2
		6:1	4	13	<1
15	SindbisGagMod.SF2 virus particles at 4 wks	100:1	5	38	<1
		25:1	4	18	<1
	pCMVKm2.GagMod.SF2 DNA at 0 wks	6:1	3	13	1

^a 20 µg

^b 10⁷ particles

20 * Challenge with recombinant vaccinia virus expressing HIV-1SF2 Gag at 3 weeks post second immunization (week 7). Spleens taken 5 days later. Ex vivo CTL assay performed by standard ⁵¹Cr release assay. Values seen represent results from 3 pooled mouse spleens per group

25 B. In vivo Immunization with Env-containing DNA and/or Sindbis particles

30 Balb/C mice were immunized intramuscularly at 0 and 4 weeks (as shown in the following table) with plasmid DNA and/or Sindbis-virus RNA-containing particles each containing gp120.modUS4 sequences. Treatment regimes and antibody titers are shown in Table 26. Antibody titers were determined by ELISA using gp120 SF2 protein to coat the plates.

35

Table 26					
	Treatment		Bleed 0	Bleed 1 (8 wks)	Bleed 2 (10 wks)
Animal	1st Immunization	2nd Immunization	1st Imm'n	2nd Imm'n	2 Wks post 2nd
EO 456	25 μ g 120mod DNA	(None)	8.3	45	309
EO 457			8.3	254	460
EO 458			8.3	8.3	93
EO 459			8.3	43	45
EO 460			8.3	8.3	274
EO 461	25 μ g 120mod DNA	25 μ g 120mod DNA	8.3	47	1502
EO 462			8.3	80	5776
EO 463			8.3	89	3440
EO 464			8.3	8.3	3347
EO 465			8.3	69	1127
EO 466	50 μ g 120mod DNA	(None)	8.3	63	102
EO 467			8.3	112	662
EO 468			8.3	94	459
EO 469			8.3	58	48
EO 470			8.3	95	355
EO 471	50 μ g 120mod DNA	50 μ g 120mod DNA	8.3	110	9074
EO 472			8.3	8.3	4897
EO 473			8.3	49	4089
EO 474			8.3	59	5280
EO 475			8.3	8.3	929
EO 476	25 μ g 120mod DNA	Sindbis/Env	8.3		653
EO 477			8.3	87	22675
EO 478			8.3	76	3869
EO 479			8.3		1004
EO 480			8.3	71	7080
EO 481	Sindbis/Env	(None)	8.3	8.3	8.3
EO 482			8.3	8.3	8.3
EO 483			8.3	78	103
EO 484			8.3	8.3	32
EO 485			8.3	76	207
EO 486	Sindbis/Env	Sindbis/Env	8.3	8.3	458
EO 487			8.3	8.3	345
EO 488			8.3	8.3	331
EO 489			8.3	103	111
EO 490			8.3	8.3	5636

As can be seen from the data presented above, all of the mice generally demonstrated substantial immunological responses by bleed number 2. For Env, the best results were obtained using either (i) 50 μ g of gp120.modUS4 DNA for the first immunization followed by a second

immunization using 50 μ g of gp120.modUS4 DNA, or (ii) 25 μ g of gp120.modUS4 DNA for the first immunization followed by a second immunization using 10^7 pfus of Sindbis.

5 The results presented above demonstrate that the Env and Gag proteins of the present invention are effective to induce an immune response using Sindbis vector systems which include the synthetic Env (e.g., gp120.modUS4) or Gag expression cassettes.

10

Example 15

Co-Transfection of Env and Gag as Monocistronic and Bicistronic Constructs

DNA constructs encoding (i) wild-type US4 and SF162 Env polypeptides, (ii) synthetic US4 and SF162 Env polypeptides (gp160.modUS4, gp160.modUS4.delV1/V2, gp160.modSF162, and gp120.modSF162.delV2), and (iii) SF2gag polypeptide (i.e., the Gag coding sequences obtained from the SF2 variant or optimized sequences corresponding to the gagSF2 -- gag.modSF2) were prepared. These monocistronic constructs were co-transfected into 293T cells in a transient transfection protocol using the following combinations: gp160.modUS4; gp160.modUS4 and gag.modSF2; gp160.modUS4.delV1/V2; gp160.modUS4.delV1/V2 and gag.modSF2; gp160.modSF162 and gag.modSF2; gp120.modSF162.delV2 and gag.modSF2; and gag.modSF2 alone.

Further several bicistronic constructs were made where the coding sequences for Env and Gag were under the control of a single CMV promoter and, between the two coding sequences, an IRES (internal ribosome entry site (EMCV IRES); Kozak, M., Critical Reviews in Biochemistry and Molecular Biology 27(45):385-402, 1992; Witherell, G.W., et al., Virology 214:660-663, 1995) sequence was

introduced after the Env coding sequence and before the
Gag coding sequence. Those constructs were as follows:
gp160.modUS4.gag.modSF2, SEQ ID NO:73 (Figure 61);
gp160.modUSF162.gag.modSF2, SEQ ID NO:74 (Figure 62);
5 gp160.modUS4.delV1/V2.gag.modSF2, SEQ ID NO:75 (Figure
63); and gp160.modSF162.delV2.gag.modSF2, SEQ ID NO:76
(Figure 64).

Supernatants from cell culture were filtered through
0.45 μ m filters then ultracentrifuged for 2 hours at
10 24,000 rpm (140,000Xg) in an SW28 rotor through a 20%
sucrose cushion. The pelleted materials were suspended
and layered on a 20-60% sucrose gradient and spun for 2
hours at 40,000 rpm (285,000Xg) in an SW41Ti rotor.
Gradients were fractionated into 1.0 ml samples. A total
15 of 9-10 fractions were typically collected from each DNA
transfection group.

The fractions were tested for the presence of the
Env and Gag proteins (across all fractions). These
results demonstrated that the appropriate proteins were
20 expressed in the transfected cells (i.e., if an Env
coding sequence was present the corresponding Env protein
was detected; if a Gag coding sequence was present the
corresponding Gag protein was detected).

Virus like particles (VLPs) were known to be present
25 through a selected range of sucrose densities. Chimeric
virus like particles (VLPs) were formed using all the
tested combinations of constructs containing both Env and
Gag. Significantly more protein was found in the
supernatant collected from the cells transfected with
30 "gp160.modUS4.delV1/V2 and gag.modSF2" than in all the
other supernatants.

Western blot analysis was also performed on sucrose
gradient fractions from each transfection. The results
show that bicistronic plasmids gave lower amounts of VLPs

than the amounts obtained using co-transfection with monocistronic plasmids.

In order to verify the production of chimeric VLPs by these cell lines the following electron microscopic analysis was carried out.

293T cells were plated at a density of 60-70% confluence in 100 mm dishes on the day before transfection. The cells were transfected with 10 μ g of DNA in transfection reagent LT1 (Panvera Corporation, 545 Science Dr., Madison, WI). The cells were incubated overnight in reduced serum medium (opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium was replaced with 10% fetal calf serum, 2% glutamine in IMDM in the morning of the next day and the cells were incubated for 65 hours. Supernatants and lysates were collected for analysis as described above (see Example 2).

The fixed, transfected 293T cells and purified ENV-GAG VLPs were analyzed by electron microscopy. The cells were fixed as follows. Cell monolayers were washed twice with PBS and fixed with 2% glutaraldehyde. For purified VLPs, gradient peak fractions were collected and concentrated by ultracentrifugation (24,000 rpm) for 2 hours. Electron microscopic analysis was performed by Prof. T.S. Benedict Yen (Veterans Affairs, Medical Center, San Francisco, CA).

Electron microscopy was carried out using a transmission electron microscope (Zeiss 10c). The cells were pre-stained with osmium and stained with uranium acetate and lead citrate. Immunostaining was performed to visualize envelope on the VLP. The magnification was 100,000X.

Figures 65A-65F show micrographs of 293T cells transfected with the following constructs: Figure 65A, gag.modSF2; Figure 65B, gp160.modUS4; Figure 65C,

gpl60.modUS4.delV1/V2.gag.modSF2 (bicistronic Env and
Gag); Figures 65D and 65E, gpl60.modUS4.delV1/V2 and
gag.modSF2; and Figure 65F, gpl20.modSF162.delV2 and
gag.modSF2. In the figures, free and budding immature
5 virus-like-particles (VLPs) of the expected size
(approximately 100 nm) decorated with the Env protein
were seen. In sum, gpl60 polypeptides incorporate into
Gag VLPs when constructs were co-transfected into cells.
The efficiency of incorporation is 2-3 fold higher when
10 constructs encoding V-deleted Env polypeptides from high
synthetic expression cassettes are used.

Although preferred embodiments of the subject
invention have been described in some detail, it is
understood that obvious variations can be made without
15 departing from the spirit and the scope of the invention
as defined by the appended claims.

What Is Claimed Is:

1. An expression cassette, comprising
5 a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the polynucleotide sequence encoding said Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:20.

10 2. The expression cassette of claim 1, comprising, a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the polynucleotide sequence encoding said Gag polypeptide
15 comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:9.

20 3. The expression cassette of claim 1, wherein said polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4.

25 4. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV protease polypeptide.

30 5. The expression cassette of claim 4, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:78, and SEQ ID NO:79.

6. The expression cassette of claim 1, wherein said

polynucleotide sequence further includes a polynucleotide sequence encoding an HIV reverse transcriptase polypeptide.

5 7. The expression cassette of claim 6, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, and SEQ
10 ID NO:84.

 8. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV tat polypeptide.
15

 9. The expression cassette of claim 8, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88 and SEQ ID NO:89.
20

 10. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV polymerase polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6.
25

 11. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV polymerase polypeptide, wherein (i) the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90%
30

sequence identity to the sequence presented as SEQ ID NO:4, and (ii) wherein the sequence is modified by deletions of coding regions corresponding to reverse transcriptase and integrase.

5

12. The expression cassette of claim 11, wherein said polynucleotide sequence preserves T-helper cell and CTL epitopes.

10

13. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HCV core polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:7.

15

14. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59).

20

15. The expression cassette of claim 14, wherein said *Env* polypeptide includes sequences flanking a V1 region but has a deletion in the V1 region itself.

25

16. The expression cassette of claim 15, wherein the polynucleotide sequence encoding the polypeptide comprises the sequence presented as SEQ ID NO:65 (Figure 52 gp160.modUS4.delV1).

30

17. The expression cassette of claim 14, wherein

said Env polypeptide includes sequences flanking a V2 region but has a deletion in the V2 region itself.

18. The expression cassette of claim 17, wherein
5 the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:60 (Figure 47); and SEQ ID NO:66 (Figure 53).

19. The expression cassette of claim 17, wherein
10 the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:34 (Figure 20); SEQ ID NO:37 (Figure 24); SEQ ID NO:40 (Figure 27); SEQ ID NO:43 (Figure 30); SEQ ID NO:46 (Figure 33); SEQ ID NO:49 (Figure 36); and SEQ ID NO:76
15 (Figure 64).

20. The expression cassette of claim 14, wherein
said Env polypeptide includes sequences flanking a V1/V2 region but has a deletion in the V1/V2 region itself.
20

21. The expression cassette of claim 20, wherein
the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:59 (Figure 46); SEQ ID NO:61 (Figure 48); SEQ ID NO:67
25 (Figure 54); and SEQ ID NO:75 (Figure 63).

22. The expression cassette of claim 20, wherein
the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:35
30 (Figure 21); SEQ ID NO:38 (Figure 25); SEQ ID NO:41 (Figure 28); SEQ ID NO:44 (Figure 31); SEQ ID NO:47 (Figure 34) and SEQ ID NO:50 (Figure 37).

23. The expression cassette of claim 14, wherein said Env polypeptide has a mutated cleavage site that prevents the cleavage of a gp140 polypeptide into a gp120 polypeptide and a gp41 polypeptide.

5

24. The expression cassette of claim 23, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:57 (Figure 44); SEQ ID NO:61 (Figure 48); and SEQ ID NO:63 (Figure 50).

10

25. The expression cassette of claim 23, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34).

15

20

26. The expression cassette of claim 14, wherein said Env polypeptide includes a gp160 Env polypeptide or a polypeptide derived from a gp160 Env polypeptide.

25

27. The expression cassette of claim 26, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:64 (Figure 51); SEQ ID NO:65 (Figure 52); SEQ ID NO:66 (Figure 53); SEQ ID NO:67 (Figure 54); SEQ ID NO:68 (Figure 55); SEQ ID NO:75 (Figure 63); and SEQ ID NO:73 (Figure 61).

30

28. The expression cassette of claim 26, wherein

the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:48 (Figure 35); SEQ ID NO:49 (Figure 36); SEQ ID NO:50 (Figure 37); SEQ ID NO:76 (Figure 64); and SEQ ID NO:74 (Figure 62).

29. The expression cassette of claim 14, wherein said Env polypeptide includes a gp140 Env polypeptide or a polypeptide derived from a gp140 Env polypeptide.

30. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:56 (Figure 43); SEQ ID NO:57 (Figure 44); SEQ ID NO:58 (Figure 45); SEQ ID NO:59 (Figure 46); SEQ ID NO:60 (Figure 47); SEQ ID NO:61 (Figure 48); SEQ ID NO:62 (Figure 49); and SEQ ID NO:63 (Figure 50).

31. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:36 (Figure 23); SEQ ID NO:37 (Figure 24); SEQ ID NO:38 (Figure 25); SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34).

32. The expression cassette of claim 14, wherein said Env polypeptide includes a gp120 Env polypeptide or a polypeptide derived from a gp120 Env polypeptide.

33. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:54 (Figure 41); and SEQ ID NO:55 (Figure 42).

5

34. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:33 (Figure 19); SEQ ID NO:34 (Figure 20); and SEQ ID NO:35 (Figure 21).

10

35. The expression cassette of claim 14, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:55 (Figure 42); SEQ ID NO:62 (Figure 49); SEQ ID NO:63 (Figure 50); and SEQ ID NO:68 (Figure 55).

15

36. A recombinant expression system for use in a selected host cell, comprising, an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected host cell.

20

37. The recombinant expression system of claim 36, wherein said control elements are selected from the group consisting of a transcription promoter, a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

30

38. The recombinant expression system of claim 36, wherein said transcription promoter is selected from the

group consisting of CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

5 39. A cell comprising an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell.

10 40. The cell of claim 39, wherein the cell is a mammalian cell.

15 41. The cell of claim 40, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.

42. The cell of claim 41, wherein said cell is a CHO cell.

20 43. The cell of claim 39, wherein the cell is an insect cell.

44. The cell of claim 43, wherein the cell is either *Trichoplusia ni* (Tn5) or Sf9 insect cells.

25 45. The cell of claim 39, wherein the cell is a bacterial cell.

30 46. The cell of claim 39, wherein the cell is a yeast cell.

47. The cell of claim 39, wherein the cell is a plant cell.

48. The cell of claim 39, wherein the cell is an antigen presenting cell.

49. The cell of claim 48, wherein the lymphoid cell is selected from the group consisting of macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof.

50. The cell of claim 39, wherein the cell is a primary cell.

51. The cell of claim 39, wherein the cell is an immortalized cell.

52. The cell of claim 39, wherein the cell is a tumor-derived cell.

53. A method for producing a polypeptide including HIV Gag polypeptide sequences, said method comprising, incubating the cells of claim 39, under conditions for producing said polypeptide.

54. A method for producing virus-like particles (VLPs), comprising, incubating the cells of claim 39, under conditions for producing said VLPs.

55. A method for producing a composition of virus-like particles (VLPs), comprising,
(a) incubating the cells of claim 39, under conditions for producing said VLPs; and
(b) substantially purifying said VLPs to produce a composition of VLPs.

56. A cell line useful for packaging lentivirus vectors, comprising

5 suitable host cells that have been transfected with an expression vector containing an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

10 57. The cell line of claim 56, wherein suitable host cells have been transfected with an expression vector containing the expression cassette of any of claims 1-13.

15 58. The cell line of claim 56, wherein suitable host cells have been transfected with an expression vector containing the expression cassette of claim 1-3.

20 59. The cell line of claim 56, wherein suitable host cells have been transfected with an expression vector containing the expression cassette of claim 14-35.

60. A gene delivery vector for use in a Mammalian subject, comprising

25 a suitable gene delivery vector for use in said subject, wherein the vector comprises an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the subject.

30 61. A method of DNA immunization of a subject, comprising,

introducing a gene delivery vector of claim 60 into said subject under conditions that are compatible with expression of said expression cassette in said subject.

62. The method of claim 61, wherein said gene delivery vector is a nonviral vector.

5 63. The method of claim 61, wherein said vector is delivered using a particulate carrier.

64. The method of claim 63, wherein said vector is coated on a gold or tungsten particle and said coated
10 particle is delivered to said subject using a gene gun.

65. The method of claim 63, wherein said vector is encapsulated in a liposome preparation.

15 66. The method of claim 61, wherein said vector is a viral vector.

67. The method of claim 66, wherein said viral
vector is a retroviral vector.
20

68. The method of claim 67, wherein said viral vector is a lentiviral vector.

69. The method of claim 61, wherein said subject is
25 a mammal.

70. The method of claim 69, wherein said mammal is a human.

30 71. A method of generating an immune response in a subject, comprising

transfecting cells of said subject a gene delivery vector of claim 60, under conditions that permit the expression of said polynucleotide and production of said

polypeptide, thereby eliciting an immunological response to said polypeptide.

5 72. The method of claim 71, wherein said vector is a nonviral vector.

73. The method of claim 72, wherein said vector is delivered using a particulate carrier.

10 74. The method of claim 73, wherein said vector is coated on a gold or tungsten particle and said coated particle is delivered to said vertebrate cell using a gene gun.

15 75. The method of claim 73, wherein said vector is encapsulated in a liposome preparation.

20 76. The method of claim 71, wherein said vector is a viral vector.

77. The method of claim 76, wherein said viral vector is a retroviral vector.

25 78. The method of claim 77, wherein said viral vector is a lentiviral vector.

79. The method of claim 71, wherein said subject is a mammal.

30 80. The method of claim 79, wherein said mammal is a human.

81. The method of claim 71, wherein said transfecting is done ex vivo and said transfected cells

are reintroduced into said subject.

82. The method of claim 71, wherein said transfecting is done in vivo in said subject.

5

83. The method of claim 71, where said immune response is a humoral immune response.

84. The method of claim 71, where said immune response is a cellular immune response.

10

85. A gene delivery vector comprising an alphavirus vector construct, wherein said alphavirus construct comprises an expression cassette according to any one of claims 1 through 35.

15

86. The gene delivery vector of claim 85, wherein the alphavirus vector construct is a cDNA vector construct.

20

87. The gene delivery vector of claim 85, wherein the alphavirus comprises a recombinant alphavirus particle preparation.

25

88. The gene delivery vector of claim 85, wherein the vector comprises a eukaryotic layered vector initiation system.

30

89. A method of stimulating an immune response in a subject comprising administering the gene delivery vector of any one of claims 85 through 88 in an amount effective to stimulate an immune response in said subject.

90. The method of claim 89, wherein the gene

delivery vector is administered intramuscularly,
intramucosally, intranasally, subcutaneously,
intradermally, transdermall, intravaginally,
intrarectally, orally or intravenously.

5

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orig.gagSF2

ATGGGTGCGAGAGCGTCGGTATTAAGCGGGGAGAATTAGATAAATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAG

AAAAATATAAGTTAAACATATGTATGGGCAAGCAGGGAGCTAGAACGATTTCGCAGTCAATCCTGGCCTGTTAGAA
Inact. 1
G G C C G C CACATCAGAAGGCTGCAGACAAATATTGGGACAGCTACAGCCATCCCTTCAGACAGGATCAGAGAACTTAGATCATTAGAA
Inact. 2
G G C CTATAATACAGTAGCAACCCTCTATTGTGTACATCAAAGGATAGATGTAAAGACACCAAGGAAGCTTTAGAGAAGATA
Inact. 3
C GC C C GGAGGAAGAGCAAAACAAAGTAAGAAAAAGGCACAGCAAGCAGCAGCTGCAGCTGGCACAGGAAACAGCAGCCAGGTC
Inact. 4
GTCC G C G

AGCCAAAATTACCCTATAGTGCAGAACCTACAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCA

TGGGTAAAAGTAGTAGAAGAAAAGGCTTTCAGCCCAGAAGTAATACCCATGTTTTTCAGCATTATCAGAAGGAGCCACC

CCACAGATTTAAACACCATGCTAAACACAGTGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACTATCAAT
Inact. 5
G CC G G T G C

GAGGAAGCTGCAGAAATGGGATAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAAATGAGAGAACCAAGG

GGAAGTGACATAGCAGGAACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTA

GGAGAAATCTATAAAAGATGGATAATCCTGGGATTAAATAAAATAGTAAGAAATGTATAGCCCTACCAGCATTCTGGAC
Inact. 6
G C G G Inact. 7
G C G C G GATAAGACAAGGACCAAAGGAACCCTTTAGAGATTATGTAGACCGGTTCTATAAACTCTAAGAGCGAACAAGCTTCA
Inact. 8
C CC G G TCAGGATGTAAAAAATTGGATGACAGAAACCTTGTTGGTCCAAAATGCAAACCCAGATTGTAAGAGTATTTTAAAGCA
Inact. 8
C CC G G TTTGGGACAGCAGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTGGGGGACCCGGCCATAAAGCAAGAGTT
C C C

TTGGCTGAAGCCATGAGCCAAGTAACAAATCCAGCTAACATAATGATGCAGAGAGGCAATTTTAGGAACCAAAGAAAG

ACTGTAAAGTGTTTCAATTGTGGCAAAGAAGGGCACATAGCCAAAAATTGCAGGGCCCCCTAGGAAAAGGGCTGTTGG

AGATGTGGAAGGGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCC

TACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGG

GAGGAGAAAACAACTCCCTCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAACTCCCTCAGATCACTC

TTTGGCAACGACCCCTCGTCACAATAA

FIG. 1

SUBSTITUTE SHEET (RULE 26)

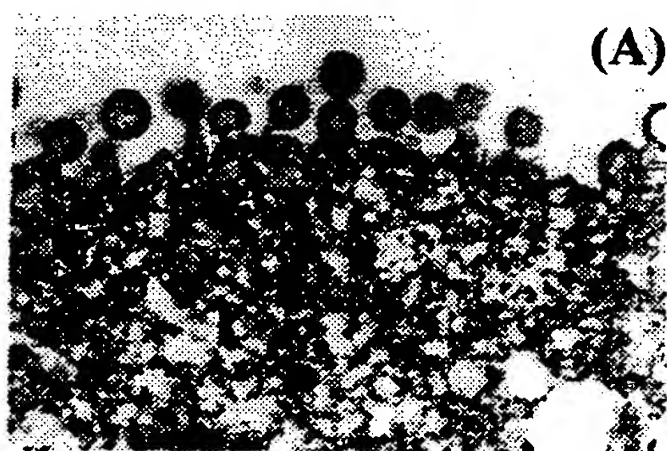


FIG. 3A

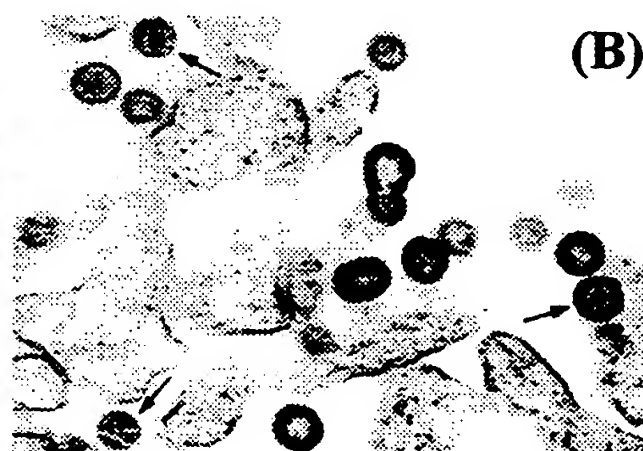


FIG. 3B

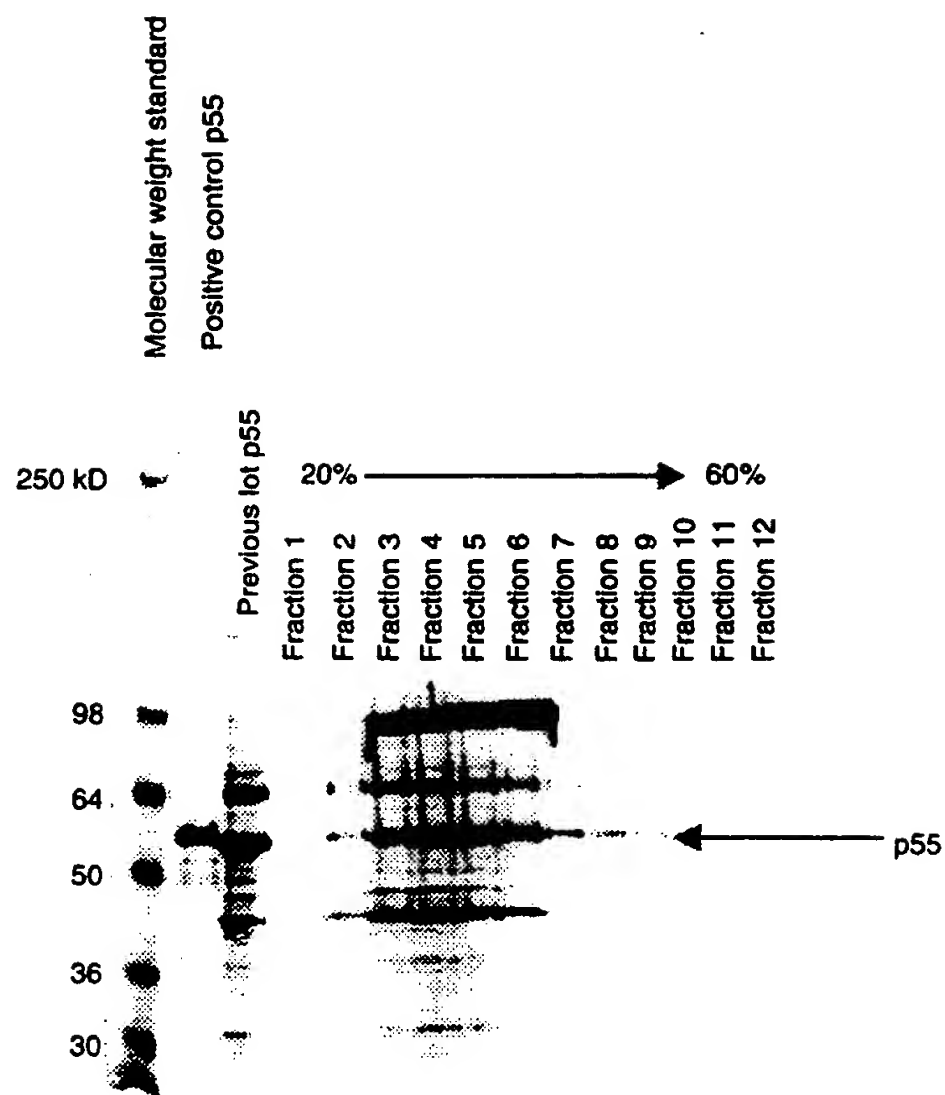


FIG. 4

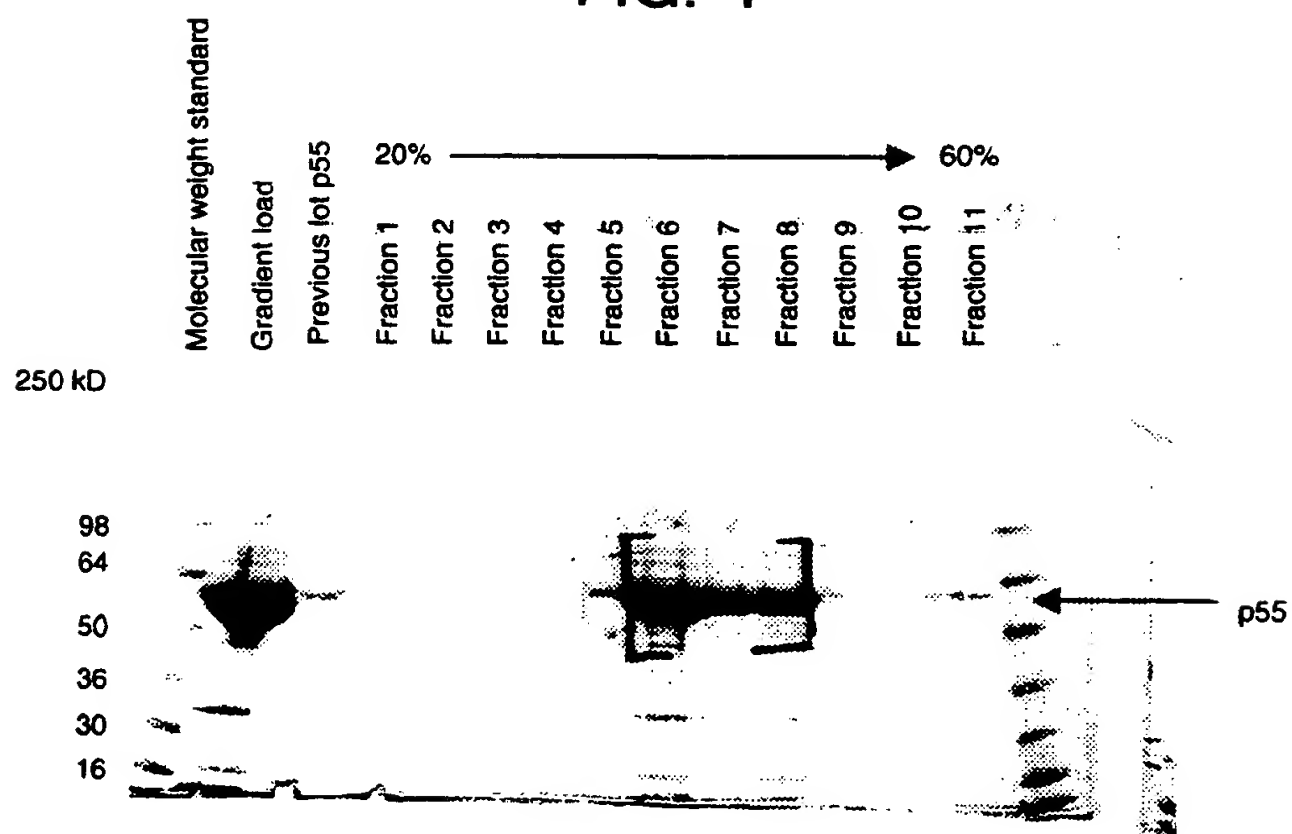


FIG. 5

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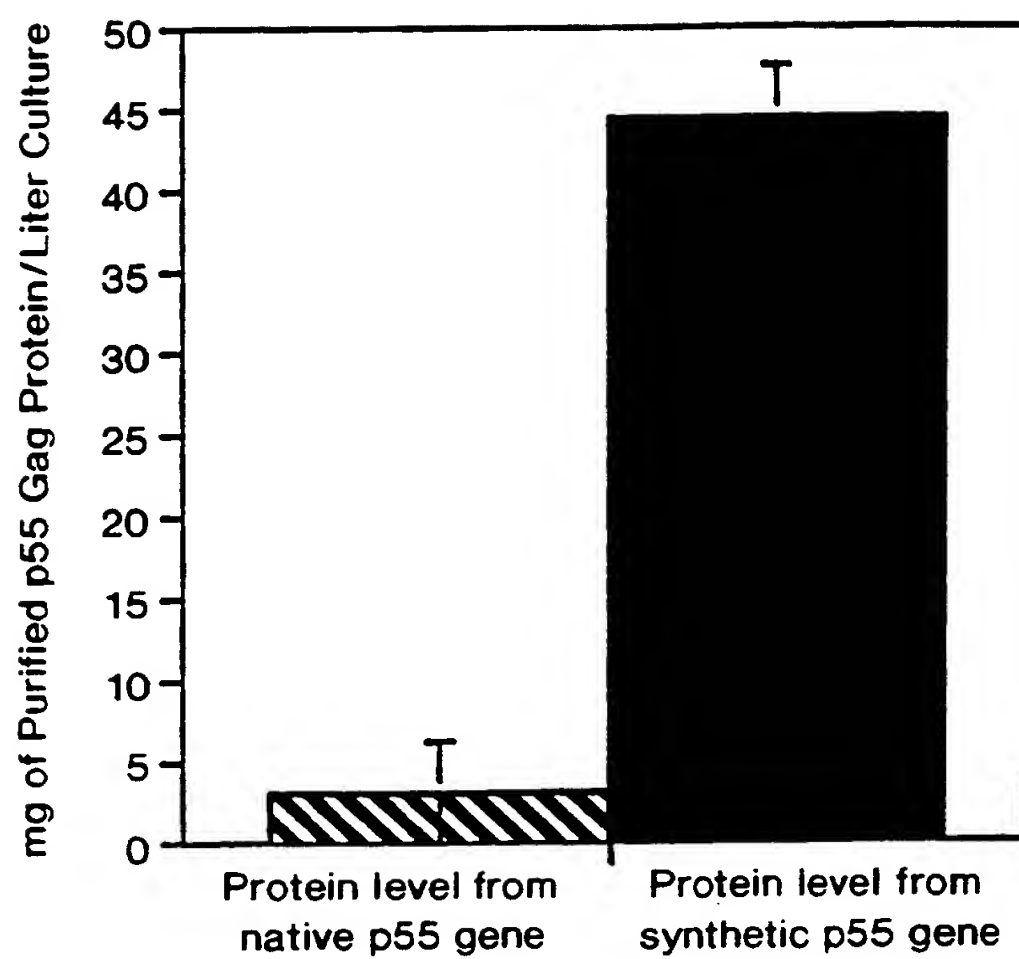


FIG. 6

GagPol.ModSF	1	ATGGGCGCCC	10	20	30	40	50
GagProt.ModS	1	ATGGGCGCCC	10	20	30	40	50
Gag.ModSF2	1	ATGGGCGCCC	10	20	30	40	50
			60	70	80	90	100
GagPol.ModSF	51	GAAGATCCGC	110	120	130	140	150
GagProt.ModS	51	GAAGATCCGC	110	120	130	140	150
Gag.ModSF2	51	GAAGATCCGC	110	120	130	140	150
GagPol.ModSF	101	TCGTGTGGC	160	170	180	190	200
GagProt.ModS	101	TCGTGTGGC	160	170	180	190	200
Gag.ModSF2	101	TCGTGTGGC	160	170	180	190	200
GagPol.ModSF	151	CTGGAGACCA	210	220	230	240	250
GagProt.ModS	151	CTGGAGACCA	210	220	230	240	250
Gag.ModSF2	151	CTGGAGACCA	210	220	230	240	250
GagPol.ModSF	201	CCTGCAGACC	260	270	280	290	300
GagProt.ModS	201	CCTGCAGACC	260	270	280	290	300
Gag.ModSF2	201	CCTGCAGACC	260	270	280	290	300
GagPol.ModSF	251	CCCTGTACTG	310	320	330	340	350
GagProt.ModS	251	CCCTGTACTG	310	320	330	340	350
Gag.ModSF2	251	CCCTGTACTG	310	320	330	340	350
GagPol.ModSF	301	CTGGAGAAGA	360	370	380	390	400
GagProt.ModS	301	CTGGAGAAGA	360	370	380	390	400
Gag.ModSF2	301	CTGGAGAAGA	360	370	380	390	400
GagPol.ModSF	351	GGCCGCCGCC	410	420	430	440	450
GagProt.ModS	351	GGCCGCCGCC	410	420	430	440	450
Gag.ModSF2	351	GGCCGCCGCC	410	420	430	440	450
GagPol.ModSF	401	ACCCCATCGT	460	470	480	490	500
GagProt.ModS	401	ACCCCATCGT	460	470	480	490	500
Gag.ModSF2	401	ACCCCATCGT	460	470	480	490	500

FIG. 7A

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GagPol.ModSF	451	460	470	480	490	500
GagProt.ModS	451	460	470	480	490	500
Gag.ModSF2	451	460	470	480	490	500
GagPol.ModSF	501	510	520	530	540	550
GagProt.ModS	501	510	520	530	540	550
Gag.ModSF2	501	510	520	530	540	550
GagPol.ModSF	551	560	570	580	590	600
GagProt.ModS	551	560	570	580	590	600
Gag.ModSF2	551	560	570	580	590	600
GagPol.ModSF	601	610	620	630	640	650
GagProt.ModS	601	610	620	630	640	650
Gag.ModSF2	601	610	620	630	640	650
GagPol.ModSF	651	660	670	680	690	700
GagProt.ModS	651	660	670	680	690	700
Gag.ModSF2	651	660	670	680	690	700
GagPol.ModSF	701	710	720	730	740	750
GagProt.ModS	701	710	720	730	740	750
Gag.ModSF2	701	710	720	730	740	750
GagPol.ModSF	751	760	770	780	790	800
GagProt.ModS	751	760	770	780	790	800
Gag.ModSF2	751	760	770	780	790	800
GagPol.ModSF	801	810	820	830	840	850
GagProt.ModS	801	810	820	830	840	850
Gag.ModSF2	801	810	820	830	840	850
GagPol.ModSF	851	860	870	880	890	900
GagProt.ModS	851	860	870	880	890	900
Gag.ModSF2	851	860	870	880	890	900

FIG. 7B

GagPol.ModSF	901	CGCTTCTACA	910	AGACCCCTGCG	920	CGCTGAGCAG	930	GCCAGCCAGG	940	ACGTGAAGAA	950
GagProt.ModS	901	CGCTTCTACA		AGACCCCTGCG		CGCTGAGCAG		GCCAGCCAGG		ACGTGAAGAA	950
Gag.ModSF2	901	CGCTTCTACA	960	AGACCCCTGCG	970	CGCTGAGCAG	980	GCCAGCCAGG	990	ACGTGAAGAA	950
GagPol.ModSF	951	CTGGATGACC		GAGACCCCTGC		TGGTGACAGAA		CGCCAAACCCC		GACTGCAAGA	1000
GagProt.ModS	951	CTGGATGACC		GAGACCCCTGC		TGGTGACAGAA		CGCCAAACCCC		GACTGCAAGA	1000
Gag.ModSF2	951	CTGGATGACC	1010	GAGACCCCTGC	1020	TGGTGACAGAA	1030	CGCCAAACCCC	1040	GACTGCAAGA	1000
GagPol.ModSF	1001	CCATCCTGAA		GGCTCTCGGC		CCCGCGGCCA		CCCTGGAGGA		GATGATGACC	1050
GagProt.ModS	1001	CCATCCTGAA		GGCTCTCGGC		CCCGCGGCCA		CCCTGGAGGA		GATGATGACC	1050
Gag.ModSF2	1001	CCATCCTGAA	1060	GGCTCTCGGC	1070	CCCGCGGCCA	1080	CCCTGGAGGA	1090	GATGATGACC	1050
GagPol.ModSF	1051	GCCTGCCAGG		GCCTGGGCGG		CCCGGGCCAC		AAGGCCCGCG		TGCTGGCCGA	1100
GagProt.ModS	1051	GCCTGCCAGG		GCCTGGGCGG		CCCGGGCCAC		AAGGCCCGCG		TGCTGGCCGA	1100
Gag.ModSF2	1051	GCCTGCCAGG	1110	GCCTGGGCGG	1120	CCCGGGCCAC	1130	AAGGCCCGCG	1140	TGCTGGCCGA	1100
GagPol.ModSF	1101	GGCGATGAGC		CAGGTGACGA		ACCCGGCGGAC		CATCATGATG		CAGCGCGGCA	1150
GagProt.ModS	1101	GGCGATGAGC		CAGGTGACGA		ACCCGGCGGAC		CATCATGATG		CAGCGCGGCA	1150
Gag.ModSF2	1101	GGCGATGAGC	1160	CAGGTGACGA	1170	ACCCGGCGGAC	1180	CATCATGATG	1190	CAGCGCGGCA	1150
GagPol.ModSF	1151	ACTTCCGCAA		CCAGCGGAAG		ACCGTCAAGT		GCTTCAACTG		CGGCAAGGAG	1200
GagProt.ModS	1151	ACTTCCGCAA		CCAGCGGAAG		ACCGTCAAGT		GCTTCAACTG		CGGCAAGGAG	1200
Gag.ModSF2	1151	ACTTCCGCAA	1210	CCAGCGGAAG	1220	ACCGTCAAGT	1230	GCTTCAACTG	1240	CGGCAAGGAG	1200
GagPol.ModSF	1201	GGCCACACCG		CCAGGAACTG		CCGCGCCCCC		CGCAAGAAGG		GCTGCTGGCG	1250
GagProt.ModS	1201	GGCCACACCG		CCAGGAACTG		CCGCGCCCCC		CGCAAGAAGG		GCTGCTGGCG	1250
Gag.ModSF2	1201	GGCCACACCG	1260	CCAGGAACTG	1270	CCGCGCCCCC	1280	CGCAAGAAGG	1290	GCTGCTGGCG	1250
GagPol.ModSF	1251	CTGCGGCCGC		GAAGGACACC		AAATGAAAGA		TTGCACTGAG		AGACAGGCTA	1300
GagProt.ModS	1251	CTGCGGCCGC		GAAGGACACC		AAATGAAAGA		TTGCACTGAG		AGACAGGCTA	1300
Gag.ModSF2	1251	CTGCGGCCGC	1310	GAAGGACACC	1320	AAATGAAAGA	1330	CTGCACCCGAG	1340	CGCCAGGCTA	1300
GagPol.ModSF	1301	ATTTTCTAGG		GAAGATCTGG		CCTTCTCTACA		AGGGAAGGCC		AGGGAATTTT	1350
GagProt.ModS	1301	ATTTTCTAGG		GAAGATCTGG		CCTTCTCTACA		AGGGAAGGCC		AGGGAATTTT	1350
Gag.ModSF2	1301	ATTTTCTAGG	1360	GAAGATCTGG	1370	CCTTCTCTACA	1380	AGGGAAGGCC	1390	AGGGAATTTT	1350

FIG. 7C

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GagPol.ModSF	1351	CTTCAGAGCA	1360	GACCAGAGCC	1370	AACAGCCCCA	1380	CCAGAAGAGA	1390	GCTTCAGGTT	1400
GagProt.ModS	1351	CTTCAGAGCA	1410	GACCAGAGCC	1420	AACAGCCCCA	1430	CCAGAAGAGA	1440	GCTTCAGGTT	1450
Gag.ModSF2	1351	CTGCAGAGCC	1460	GCCCCGAGCC	1470	CACCGCCCCC	1480	CCCGAGGAGA	1490	GCTTCGCGTT	1500
GagPol.ModSF	1401	TGGGAGGAG	1510	AAACAACATC	1520	CCTCTCAGAA	1530	GCAGGAGCCG	1540	ATAGACAAGG	1550
GagProt.ModS	1401	TGGGAGGAG	1560	AAACAACATC	1570	CCTCTCAGAA	1580	GCAGGAGCCG	1590	ATAGACAAGG	1600
Gag.ModSF2	1401	CGGCGAGGAG	1610	AAGACCAACC	1620	CCAGCCAGAA	1630	GCAGGAGCCG	1640	ATCGACAAGG	1650
GagPol.ModSF	1451	AACTGTATCC	1660	TTTAACTTCC	1670	CTCAGATCAC	1680	TCTTTGGCAA	1690	CGACCCCTCG	1700
GagProt.ModS	1451	AACTGTATCC	1710	TTTAACTTCC	1720	CTCAGATCAC	1730	TCTTTGGCAA	1740	CGACCCCTCG	1750
Gag.ModSF2	1451	AGCTGTATCC	1760	CCTGACCCAGC	1770	CTGCGCAGCC	1780	TGTTCCGGCAA	1790	CGACCCCTCG	1800
GagPol.ModSF	1501	TCACAGTAAG	1810	GATCGGCGGC	1820	CAGCTCAAGG	1830	AGGCGCTGCT	1840	CGACACCCGGC	1850
GagProt.ModS	1501	TCACAGTAAG	1860	GATCGGCGGC	1870	CAGCTCAAGG	1880	AGGCGCTGCT	1890	CGACACCCGGC	1900
Gag.ModSF2	1501	AGCCAGTAA	1910	1920	1930	1940	1950
GagPol.ModSF	1551	GCCGACGACA	1960	CCGTGCTGGA	1970	GGAGATGAAC	1980	CTGCCCGGCA	1990	AGTGGAAAGC	2000
GagProt.ModS	1551	GCCGACGACA	2010	CCGTGCTGGA	2020	GGAGATGAAC	2030	CTGCCCGGCA	2040	AGTGGAAAGC	2050
Gag.ModSF2	1551	2060	2070	2080	2090	2100
GagPol.ModSF	1601	CAAGATGATC	2110	GGCGGGATCG	2120	GGGGCTTCAT	2130	CAAGGTGCGG	2140	CAGTACGACC	2150
GagProt.ModS	1601	CAAGATGATC	2160	GGCGGGATCG	2170	GGGGCTTCAT	2180	CAAGGTGCGG	2190	CAGTACGACC	2200
Gag.ModSF2	1601	2210	2220	2230	2240	2250
GagPol.ModSF	1651	AGATCCCCGT	2260	GGAGATCTGC	2270	GGCCACAAGG	2280	CCATCGGCAC	2290	CGTGCTGGTG	2300
GagProt.ModS	1651	AGATCCCCGT	2310	GGAGATCTGC	2320	GGCCACAAGG	2330	CCATCGGCAC	2340	CGTGCTGGTG	2350
Gag.ModSF2	1651	2360	2370	2380	2390	2400
GagPol.ModSF	1701	GGCCCCACCC	2410	CCGTGAACAT	2420	CATCGGCCGC	2430	AACCTGCTGA	2440	CCCAGATCGG	2450
GagProt.ModS	1701	GGCCCCACCC	2460	CCGTGAACAT	2470	CATCGGCCGC	2480	AACCTGCTGA	2490	CCCAGATCGG	2500
Gag.ModSF2	1701	2510	2520	2530	2540	2550
GagPol.ModSF	1751	CTGCACCCCTG	2560	AACTTCCCCA	2570	TCAGCCCCAT	2580	CGAGACGGTG	2590	CCCGTGAAGC	2600
GagProt.ModS	1751	CTGCACCCCTG	2610	AACTTCCCCA	2620	TCAGCCCCAT	2630	CGAGACGGTG	2640	CCCGTGAAGC	2650
Gag.ModSF2	1751	2660	2670	2680	2690	2700

FIG. 7D

		1810		1820		1830		1840		1850	
GagPol.ModSF	1801	TGAAGCCGGG	GATGGACGGC	CCCAAGGTCA	AGCAGTGGCC	CCTGACCGAG	1850				
	1801	TGAAGCCGGG	GATGGACGGC	CCCAAGGTCA	AGCAGTGGCC	CCTGTAA...	1850				
	1801	1850				
	1801	1850				
GagPol.ModSF	1851	GAGAAGATCA	AGGCCCTGGT	GGAGATCTGC	ACCGAGATGG	AGAAGGAGGG	1900				
	1851	1900				
	1851	1900				
	1851	1900				
GagPol.ModSF	1901	CAAGATCAGC	AAGATCGGCC	CCGAGAACCC	CTACAACACC	CCCGTGTTCG	1950				
	1901	1950				
	1901	1950				
	1901	1950				
GagPol.ModSF	1951	CCATCAAGAA	GAAGGACAGC	ACCAAGTGGC	GCAAGCTGGT	GGACTTCCGC	2000				
	1951	2000				
	1951	2000				
	1951	2000				
GagPol.ModSF	2001	GAGCTGAACA	AGCGCACCCA	GGACTTCTGG	GAGGTGCAGC	TGGGCATCCC	2050				
	2001	2050				
	2001	2050				
	2001	2050				
GagPol.ModSF	2051	CCACCCCGCC	GGCCTGAAGA	AGAAGAAGAG	CGTGACCGTG	CTGGACGTGG	2100				
	2051	2100				
	2051	2100				
	2051	2100				
GagPol.ModSF	2101	GCGACGCCTA	CTTCAGCGTG	CCCCCTGGACA	AGGACTTCCG	CAAGTACACC	2150				
	2101	2150				
	2101	2150				
	2101	2150				
GagPol.ModSF	2151	GCCTTCACCA	TCCCCAGCAT	CAACAACGAG	ACCCCCGGCA	TCCGCTACCA	2200				
	2151	2200				
	2151	2200				
	2151	2200				
GagPol.ModSF	2201	GTACAAACGTG	CTGCCCCCAGG	GCTGGAAGGG	CAGCCCCGCC	ATCTTCCAGA	2250				
	2201	2250				
	2201	2250				
	2201	2250				

FIG. 7E

GagPol.ModSF	2251	2260	2270	2280	2290	2300
GagProt.ModS	2251	GCAGCATGAC	CAAGATCCTG	GAGCCCTTCC	GCAAGCAGAA	CCCCGACATC
Gag.ModSF2	2251
		2310	2320	2330	2340	2350
GagPol.ModSF	2301	GTGATCTACC	AGTACATGGA	CGACCTGTAC	GTGGGCAGCG	ACCTGGAGAT
GagProt.ModS	2301
Gag.ModSF2	2301
		2360	2370	2380	2390	2400
GagPol.ModSF	2351	CGGCCAGCAC	CGCACCAAGA	TCGAGGAGCT	GCGCCAGCAC	CTGCTGCGCT
GagProt.ModS	2351
Gag.ModSF2	2351
		2410	2420	2430	2440	2450
GagPol.ModSF	2401	GGGGCTTCAC	CACCCCCGAC	AAGAAGCACC	AGAAGGAGCC	CCCCTTCCCTG
GagProt.ModS	2401
Gag.ModSF2	2401
		2460	2470	2480	2490	2500
GagPol.ModSF	2451	TGGATGGGCT	ACGAGCTGCA	CCCCGACAAG	TGGACCGTGC	AGCCCATCAT
GagProt.ModS	2451
Gag.ModSF2	2451
		2510	2520	2530	2540	2550
GagPol.ModSF	2501	GCTGCCCCGAG	AAGGACAGCT	GGACCGTGAA	CGACATCCAG	AAGCTGGTGG
GagProt.ModS	2501
Gag.ModSF2	2501
		2560	2570	2580	2590	2600
GagPol.ModSF	2551	GCAAGCTGAA	CTGGGCCAGC	CAGATCTACG	CCGGCATCAA	GGTGAAGCAG
GagProt.ModS	2551
Gag.ModSF2	2551
		2610	2620	2630	2640	2650
GagPol.ModSF	2601	CTGTGCAAGC	TGCTGGCGGG	CACCAAGGCC	CTGACCGAGG	TGATCCCCCT
GagProt.ModS	2601
Gag.ModSF2	2601
		2660	2670	2680	2690	2700
GagPol.ModSF	2651	GACCGAGGAG	GCCGAGCTGG	AGCTGGCCGA	GAACCGGAG	ATCCTGAAGG
GagProt.ModS	2651
Gag.ModSF2	2651

FIG. 7F

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GagPol.ModSF	2701	AGCCCGTGCA	CGAGGTGTAC	TACGACCCCA	GCAAGGACCT	GGTGGCCGAG	2750
GagProt.ModS	2701	2750
Gag.ModSF2	2701	2750
GagPol.ModSF	2751	ATCCAGAAAGC	AGGGCCAGGG	CCAGTGGACC	TACCAGATCT	ACCAGGAGCC	2800
GagProt.ModS	2751	2800
Gag.ModSF2	2751	2800
GagPol.ModSF	2801	CTTCAAGAAC	CTGAAGACCG	GCAAGTACGC	CCGCATGCGC	GGCGCCCAACA	2850
GagProt.ModS	2801	2850
Gag.ModSF2	2801	2850
GagPol.ModSF	2851	CCAACGACGT	GAAGCAGCTG	ACCGAGGCCG	TGCAGAAAGT	GAGCACCCGAG	2900
GagProt.ModS	2851	2900
Gag.ModSF2	2851	2900
GagPol.ModSF	2901	AGCATCGTGA	TCTGGGGCAA	GATCCCCAAG	TTCAAGCTGC	CCATCCAGAA	2950
GagProt.ModS	2901	2950
Gag.ModSF2	2901	2950
GagPol.ModSF	2951	GGAGACCTGG	GAGGCCTGGT	GGATGGAGTA	CTGGCAGGCC	ACCTGGATCC	3000
GagProt.ModS	2951	3000
Gag.ModSF2	2951	3000
GagPol.ModSF	3001	CCGAGTGGGA	GTTCTGTGAAC	ACCCCCCCCC	TGGTGAAGCT	GTGGTACCAG	3050
GagProt.ModS	3001	3050
Gag.ModSF2	3001	3050
GagPol.ModSF	3051	CTGGAGAAGG	AGCCCCATCGT	GGGGCCCGAG	ACCTTCTACG	TGGACGGCGC	3100
GagProt.ModS	3051	3100
Gag.ModSF2	3051	3100
GagPol.ModSF	3101	CGCCAACCGC	GAGACCAAGC	TGGGCAAGGC	CGGCTACGTG	ACCGACCGCG	3150
GagProt.ModS	3101	3150
Gag.ModSF2	3101	3150

FIG. 7G

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GagPol.ModSF	3151	3160	3170	3180	3190	3200
GagProt.ModS	3151	GGCGCCAGAA	GGTGGTGAGC	ATCGCCGACA	CCACCAACCA	GAAGACCCGAG
Gag.ModSF2	3151
		3210	3220	3230	3240	3250
GagPol.ModSF	3201	CTGCAGGCCA	TCCACCTGGC	CCTGCAGGAC	AGCGGCCTGG	AGGTGAACAT
GagProt.ModS	3201
Gag.ModSF2	3201
		3260	3270	3280	3290	3300
GagPol.ModSF	3251	CGTGACCGAC	AGCCAGTACG	CCCTGGGCAT	CATCCAGGCC	CAGCCCCGACA
GagProt.ModS	3251
Gag.ModSF2	3251
		3310	3320	3330	3340	3350
GagPol.ModSF	3301	AGAGCGAGAG	CGAGCTGGTG	AGCCAGATCA	TCGAGCAGCT	GATCAAGAAG
GagProt.ModS	3301
Gag.ModSF2	3301
		3360	3370	3380	3390	3400
GagPol.ModSF	3351	GAGAAGGTGT	ACCTGGCCTG	GGTGCCCGCC	CACAAGGGCA	TCGGCGGCAA
GagProt.ModS	3351
Gag.ModSF2	3351
		3410	3420	3430	3440	3450
GagPol.ModSF	3401	CGAGCAGGTG	GACAAGCTGG	TGAGCGCCGG	CATCCGCAAG	GTGCTGTTCC
GagProt.ModS	3401
Gag.ModSF2	3401
		3460	3470	3480	3490	3500
GagPol.ModSF	3451	TGAACGGCAT	CGACAAGGCC	CAGGAGGAGC	ACGAGAAGTA	CCACAGCAAC
GagProt.ModS	3451
Gag.ModSF2	3451
		3510	3520	3530	3540	3550
GagPol.ModSF	3501	TGGCGCGCCA	TGGCCAGCGA	CTTCAACCTG	CCCCCCGTGG	TGGCCAAGGA
GagProt.ModS	3501
Gag.ModSF2	3501
		3560	3570	3580	3590	3600
GagPol.ModSF	3551	GATCGTGGCC	AGCTGGGACA	AGTGCCAGCT	GAAGGGCGAG	GCCATGCACG
GagProt.ModS	3551
Gag.ModSF2	3551

FIG. 7H

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GagPol.ModSF	3601	GCCAGGTGGA	3610	CTGCAGCCCC	3620	GGCATCTGGC	3630	AGCTGGACTG	3640	CACCCACCTG	3650
GagProt.ModS	3601	3650
Gag.ModSF2	3601	3650
GagPol.ModSF	3651	GAGGGCAAGA	3660	TCACTCTGGT	3670	GGCCGTGCAC	3680	GTGGCCAGCG	3690	GCTACATCGA	3700
GagProt.ModS	3651	3700
Gag.ModSF2	3651	3700
GagPol.ModSF	3701	GGCCGAGGTG	3710	ATCCCCGCCG	3720	AGACCCGGCCA	3730	GGAGACCGCC	3740	TACTTCCTGC	3750
GagProt.ModS	3701	3750
Gag.ModSF2	3701	3750
GagPol.ModSF	3751	TGAAGCTGGC	3760	CGGCCGCTGG	3770	CCCGTGAAGA	3780	CCATCCACAC	3790	CGACAACGGC	3800
GagProt.ModS	3751	3800
Gag.ModSF2	3751	3800
GagPol.ModSF	3801	AGCAACTTCA	3810	CCAGCACAC	3820	CGTGAAGGCC	3830	GCCTGCTGGT	3840	GGGCCGGCAT	3850
GagProt.ModS	3801	3850
Gag.ModSF2	3801	3850
GagPol.ModSF	3851	CAAGCAGGAG	3860	TTCGGCATCC	3870	CCTACAACCC	3880	CCAGAGCCAG	3890	GGCGTGGTGG	3900
GagProt.ModS	3851	3900
Gag.ModSF2	3851	3900
GagPol.ModSF	3901	AGAGCATGAA	3910	CAACGAGCTG	3920	AAGAAGATCA	3930	TCGGCCAGGT	3940	GGCGGACCAG	3950
GagProt.ModS	3901	3950
Gag.ModSF2	3901	3950
GagPol.ModSF	3951	GCCGAGCACC	3960	TGAAGACCGC	3970	CGTGCAGATG	3980	GCCGTGTTCA	3990	TCCACAACCTT	4000
GagProt.ModS	3951	4000
Gag.ModSF2	3951	4000
GagPol.ModSF	4001	CAAGCGCAAG	4010	GGCGGCATCG	4020	GCGGCTACAG	4030	CGCCGGCGAG	4040	CGCATCGTGG	4050
GagProt.ModS	4001	4050
Gag.ModSF2	4001	4050

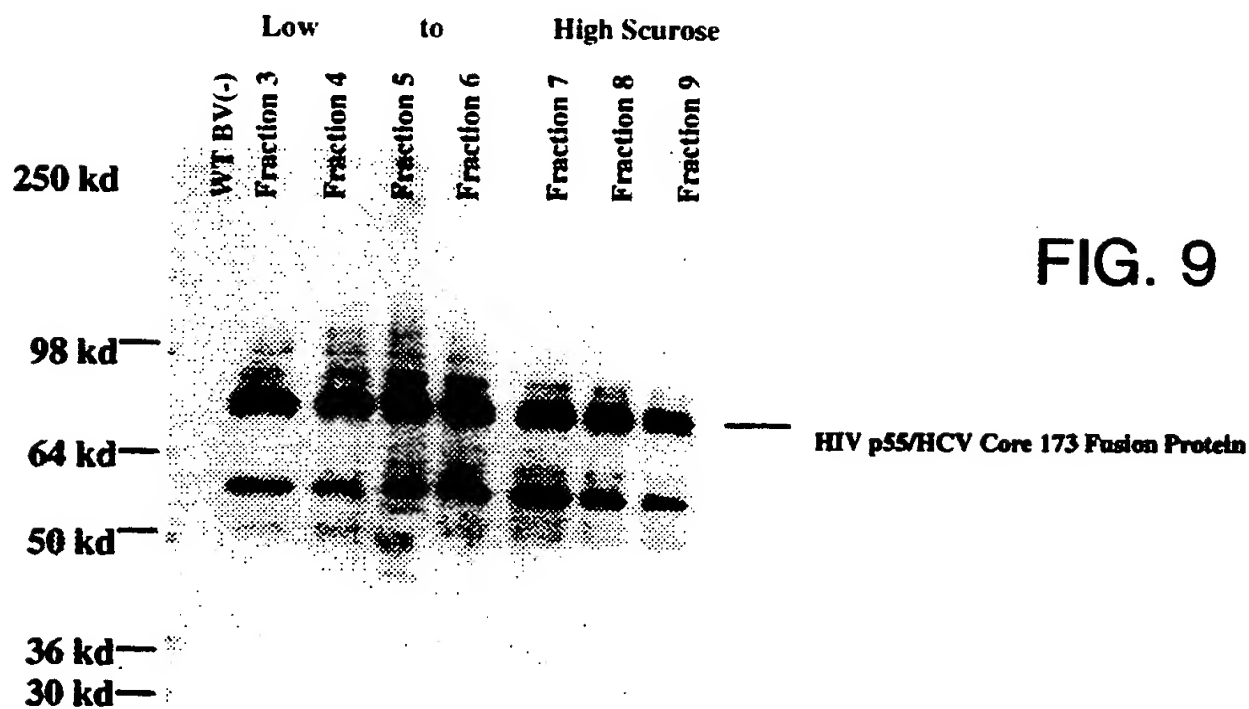
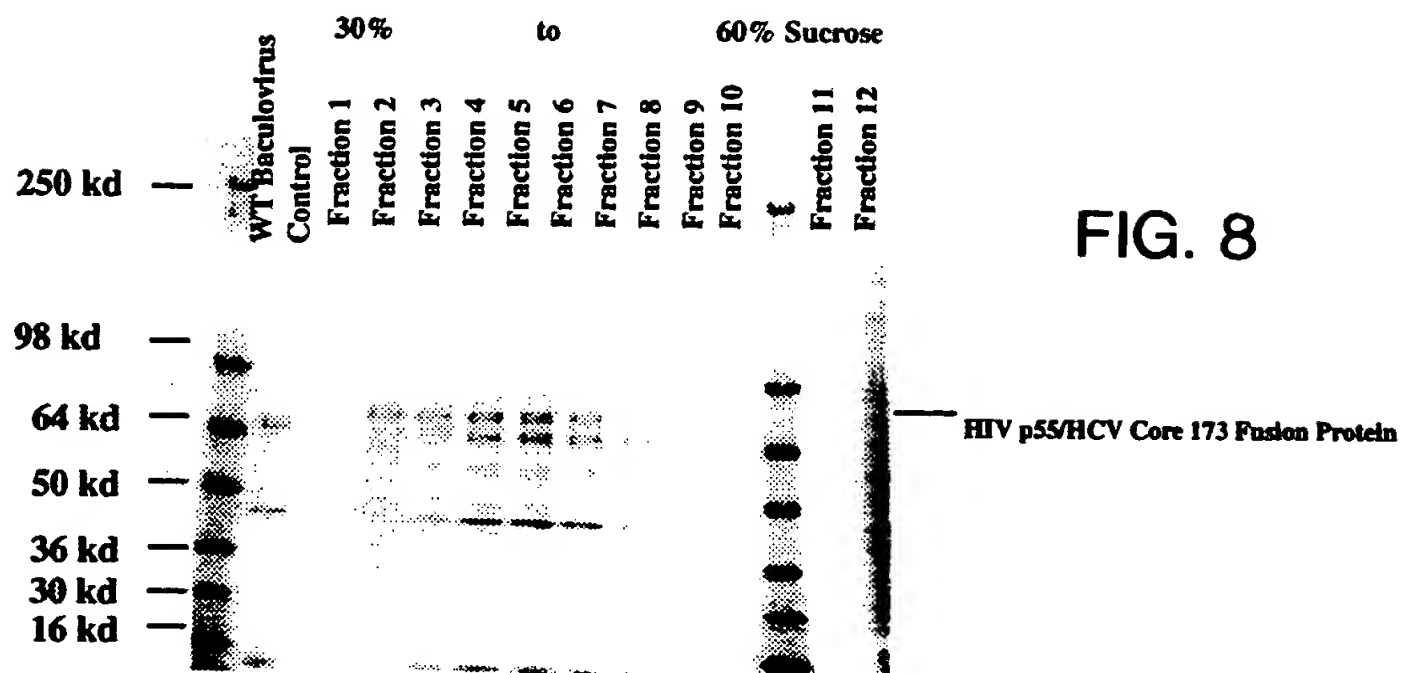
FIG. 7I

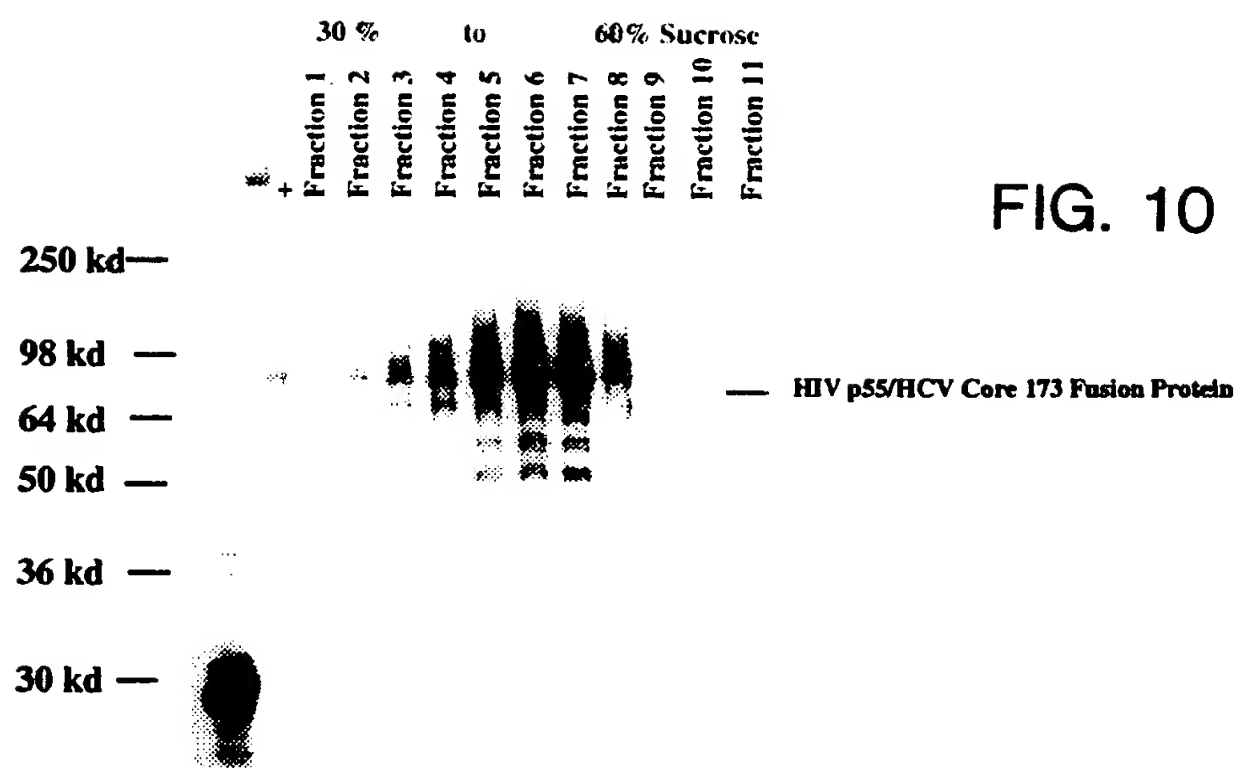
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GagPol.ModSF	4051	4060	4070	4080	4090	4100	4100
GagProt.ModS	4051	ACATCATCGC	CACCGACATC	CAGACCAAGG	AGCTGCAGAA	GCAGATCACC	4100
Gag.ModSF2	4051	4100
GagPol.ModSF	4101	4110	4120	4130	4140	4150	4150
GagProt.ModS	4101	AAGATCCAGA	ACTTCCGCGT	GTACTACCGC	GACAACAAGG	ACCCCTGTG	4150
Gag.ModSF2	4101	4150
GagPol.ModSF	4151	4160	4170	4180	4190	4200	4200
GagProt.ModS	4151	GAAGGGCCCC	GCCAAGCTGC	TGTGGAAGGG	CGAGGGCGCC	GTGGTGATCC	4200
Gag.ModSF2	4151	4200
GagPol.ModSF	4201	4210	4220	4230	4240	4250	4250
GagProt.ModS	4201	AGGACAACAG	CGACATCAAG	GTGGTGCCCC	GCCGCAAGGC	CAAGATCATC	4250
Gag.ModSF2	4201	4250
GagPol.ModSF	4251	4260	4270	4280	4290	4300	4300
GagProt.ModS	4251	CGCGACTACG	GCAAGCAGAT	GGCCGGCGAC	GACTGCGTGG	CCAGCCGCCA	4300
Gag.ModSF2	4251	4300
GagPol.ModSF	4301	4310	4320	4330	4340	4350	4350
GagProt.ModS	4301	GGACGAGGAC	TAG.....	4350
Gag.ModSF2	4301	4350

FIG. 7J

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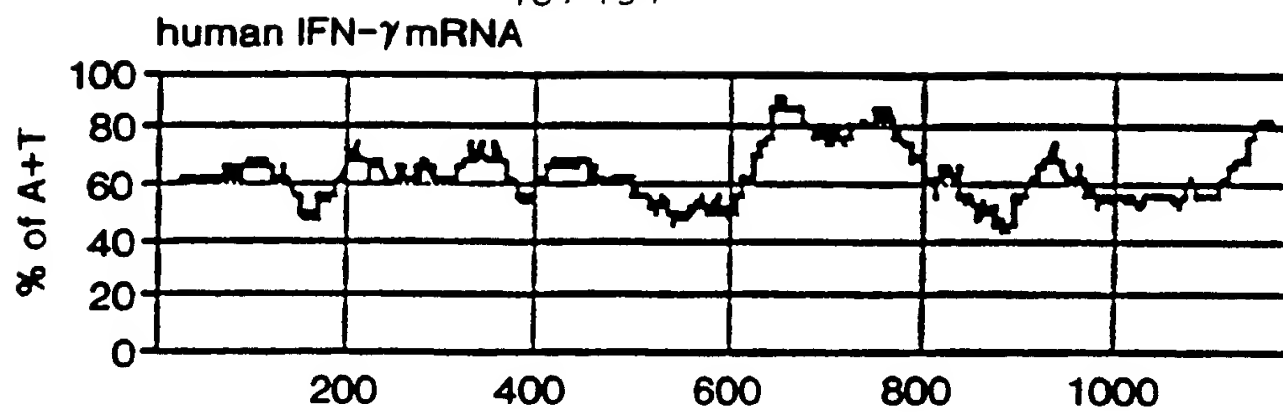


FIG. 11A

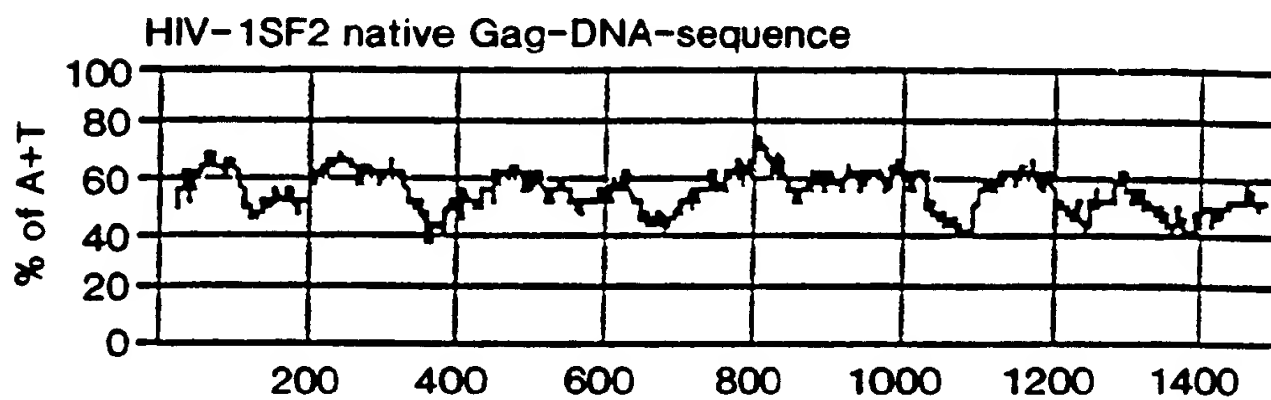


FIG. 11B

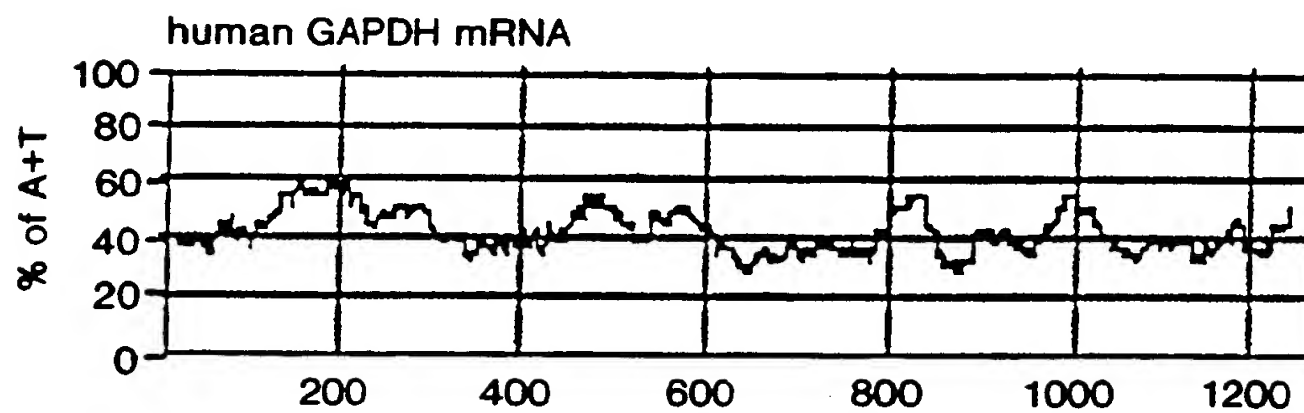


FIG. 11C

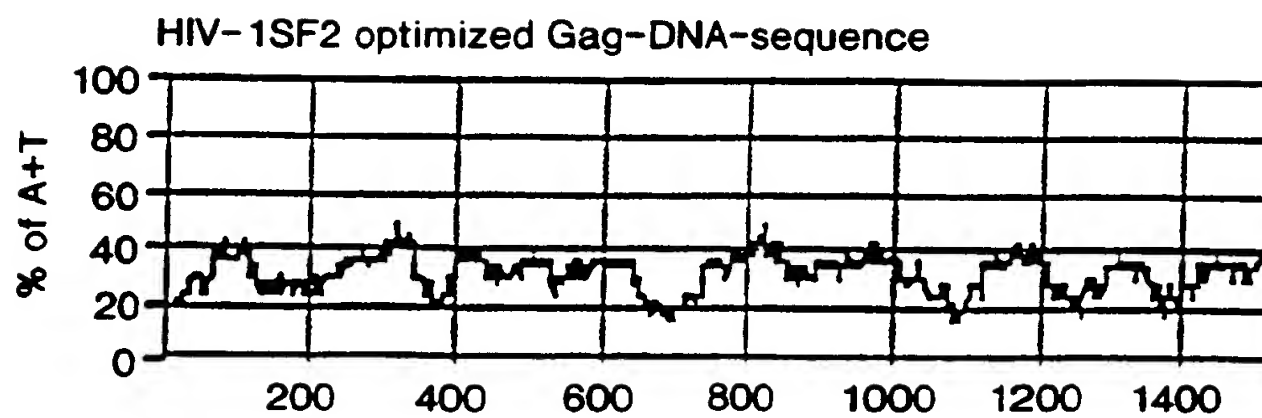


FIG. 11D

native HIV-1SF2 gag-polymerase

ATGGGTCCGAGAGCGTCGGTATTAAAGCGGGGAGAGATTAGATAAATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAG

Inact.1
AAAAATATAAGTTAAACATATAGTATGGCAAGCAGGAGCTAGAACGATTCCGAGTCAATCCTGGCCTGTTAGAA
G G C C G C C

ACATCAGAAGGCTGCAGACAAATATTGGACAGCTACAGCCATCCCTTCAGACAGGATCAGAAAGAACTTAGATCATTAGAA
Inact.2
G G C C G C C

Inact.2
TATAATACAGTAGCAACCCCTCTATTGTGTACATCAAAAGGATAGATGTAAAGACACCCAAAGGAACTTTAGAGAAAGATA
C
Inact.3
C GC C C G

GAGGAAGAGCAAAACAAAGTAAGAAAAAGGCACAGCAAGCAGCAGCTGCAGCTGGCACAGGAAACAGCAGCCAGGTC
Inact.4
GTCC G C G

AGCCAAAATTACCCCTATAGTGCAGAACCTACAGGGGGCAAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCA

TGGGTAAAGTAGTAGAAGAAAGGCTTTCAGCCCCAGAAAGTAATACCCATGTTTTTCAGCATTATCAGAAGGAGGCCACC

Inact.5
CCACAGATTTAAACACCCATGCTAAACACAGTGGGGGACATCAAGCAGCCATGCAAAATGTTAAAAAGAGACTATCAAT
G CC G G T G C

GAGGAAGCTCGAGAATGGGATAGAGTGCATCCAGTGCATGCAGGGCCCTATTGCACCCAGGCCAAATGAGAGAACCAAGG

GGAAGTGACATAGCAGGAACCTACTAGTACCCCTTCAGGAACAAATAGGATGGATGACAAATATCCACCTATCCCCAGTA

Inact.6
GGAGAAATCTATAAAAGATGGATAATCCTGGGATTAAATAAAATAGTAAGAAATGTATAGCCCTACCAGCATTTCTGGAC
G C G G G C G C G G

ATAAGACAAGGACCAAGGAACCCCTTTAGAGATTATGTAGACCGGTTCTATATAAACTCTAAGAGCCGGAACAAGCTTCA

FIG. 12A

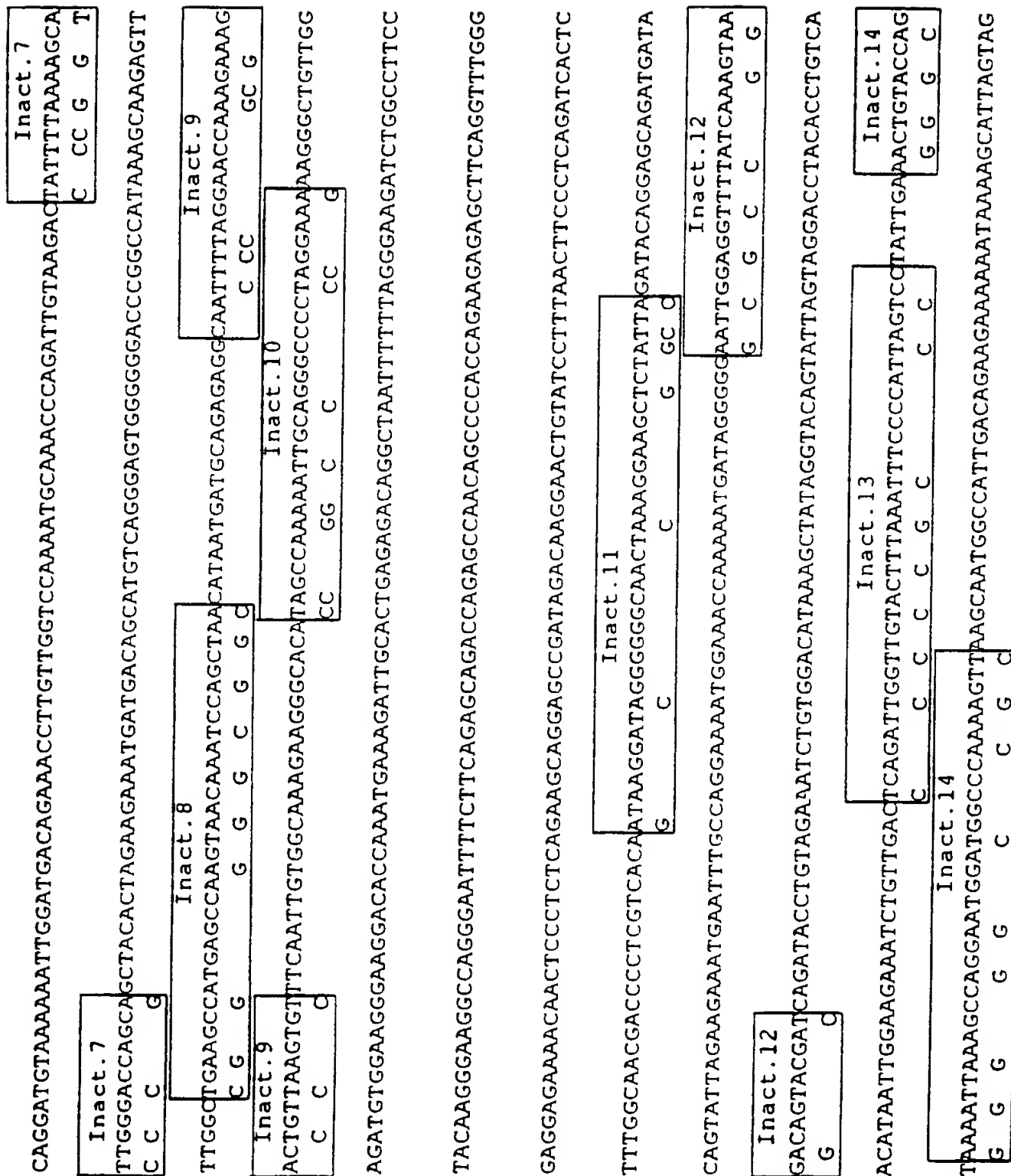


FIG. 12B

AGATATGTACAGAAATGGAAAAAGGAAGGAAAAATTTCAAAAATTTGGGCTTGAAAAATCCATACATACTCCAGTATTTG
CTATAAGAAAAAGACAGTACTAAATGGAGAAAAACTAGTAGATTTTCAGAGAACTTAATAAAGAACTCAAGACTTCT
GGGAAGTTTCAGTTAGGAATACACACCCCGAGGTTAAAAAGAAAAATCAGTAACAGTATTGGGATGTGGGTGATG
CATACTTTTCAGTTCCCTTAGATAAAAGACTTTAGAAAAGTATACTGCATTTACCATACCTAGTATAAAACAATGAGACAC
CAGGGATTAGATATCAGTACAAATGTGCTGCCACAGGGATGGAAGGATCACCAGCAATATTCCAAAGTAGCATGACAA
AAATCTTAGAGCCTTTTAGAAAAACAGAAATCCAGACATAGTTATCTATCAATACATGGATGATTGTATGTAGGATCTG
ACTTAGAAAATAGGCAGCATAGAACAAAAATAGAGAACTGAGACAGCACTCTGTTGAGGTGGGATTTACCAACACCAG
ACAAAAAACATCAGAAAAGAACCTCCATTTCTTTGGATGGTTATGAACCTCCATCCTGATAAATGGACAGTACAGCCTA
TAATGCTGCCAGAAAAAGACACAGCTGGACTGTCAATGACATACAGAAAGTTAGTGGGAAAAATTTGAATTTGGGCAAGTCAGA
TTTTATGCAGGGATTAAAGTAAAGCAGTTATGTAACTCCTTAGAGGAACCAAGCACTAACAGAACTAATACCACTAA
CAGAGAAGCAGAGCTAGAACTGGCAGAAAAACAGGGAGATTCTAAAAAGAACCACTACATGAAGTATATTATGACCCAT
CAAAAGACTTAGTAGCAAAATACAGAAAGCAGGGCAAGGCCAATGGACATATCAAAATTTATCAAGAGCCATTTTAAAA
ATCTGAAAAACAGGAAAGTATGCAAGGATGAGGGGTGCCCACTAATGATGTAAAAACAGTTAACAGAGGCAAGTGCAAA
AAGTATCCACAGAAAGCATAGTAATATGGGAAAGATTCTTAAATTTAACTACCCATACAAAAAGGAAACATGGGAAG
CATGGTGGATGGAGTATTGGCAAGCTACCTGGATTCTCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAATTTAT
GGTACCAGTTAGAGAAAGAACCCATAGTAGGAGCAGAACTTTCTATGTAGATGGGCAGCTAATAGGGAGACTAAAT
TAGGAAAAAGCAGGATATGTTACTGACAGAGGAAGACAAAAAGTTGTCTCCATAGCTGACACAAACAATCAGAAAGACTG
AATTACAAGCAATTCATCTAGCTTTGCAGGATTTCGGGATTAGAAAGTAAACATAGTAACAGACTCACAATATGCATTAG
GAATCATTCAGGACACACCATAGATAGAGTGAATCAGAGTTAGTCAGTCAATAATAGAGCAGTTAATAAANAAGGAAA
AGGTCTACCTGGCATGGGTACCAGCACACAAAGGAATTTGGAGGAAATGAACAAAGTAGATAAATTAGTCAGTGTGGAA
TCAGGAAAGTACTATTTTGAATGGAATAGATAAGGCCCAAGAGAAACATGAGAAATATCACAGTAATTTGGAGAGCAA
TGGCTAGTGATTTTAACCTGCCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAAGGAG
AAGCCATGCATGGACAAAGTAGACTGTAGTCCAGGAATATGGCACTAGATTGTACACATCTAGAAAGGAAAAATTTATCC
TGGTAGCAGTTTCATGTAGCCAGTGGATATATAGAAAGCAGAAAGTTATTCAGCAGAGACAGGGCAGGAAACAGCATATT
TTCCTTTAAAAATTAGCAGGAAGATGGCCAGTAAAAACAATACATACAGACAATGGCAGCAATTTTACCAGTACTACGG
TTAAGGCCGCCCTGTTGGTGGCAGGGATCAAGCAGGAATTTGGCATTTCCCTACAATCCCCAAAGTCAAGGAGTAGTAG
AATCTATGAATAATGAATTAAGAAAAATTTATAGGACAGGTAAAGAGATCAGGCTGAACACCTTTAAGACAGCAGTACAAA
TGGCAGTATTCATCCACAATTTTAAAAAGAAAAAGGGGATTGGGGATACAGTGCAGGGGAAAGATAGTAGACATAA
TAGCAACAGACATACAAACTAAAGAACTACAAAAGCAAAATTACAAAATTTTCGGGTTTATTACAGGGACA
ACAAAGATCCCTTTGGAAAGGACCAAGCAAGCTTCTCTGGAAAGGTGAAGGGCAGTAGTAATACAAAGATAATAGTG
ACATAAAAGTAGTGCCAAAGAAAGAAAAATCATTAGGGATTATGGGAATATGGAAAAACAGATGCGAGGTGATGATTGTGTGG
CAAGTAGACAGGATGAGGATTAG

FIG. 12C

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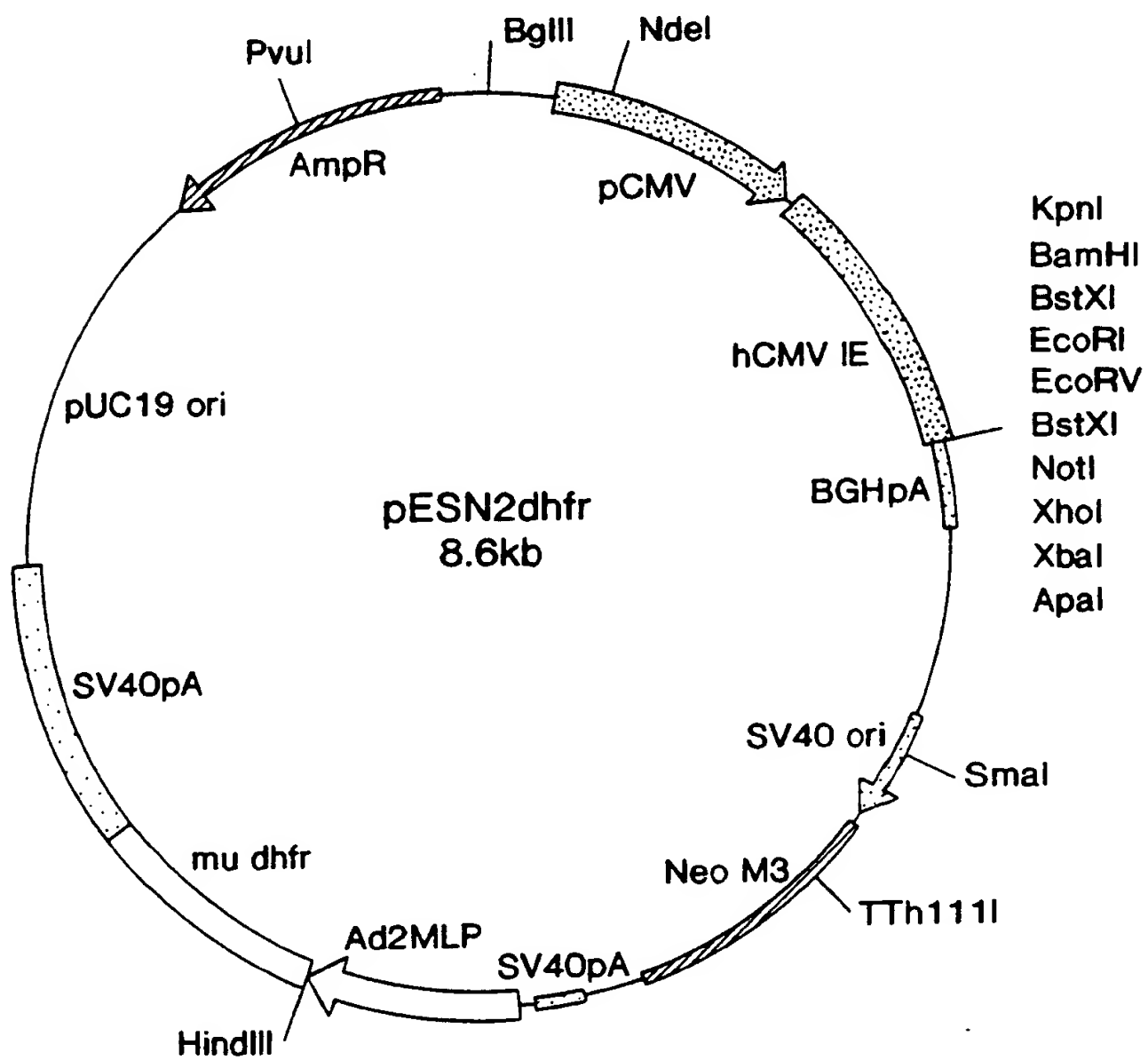


FIG. 13A

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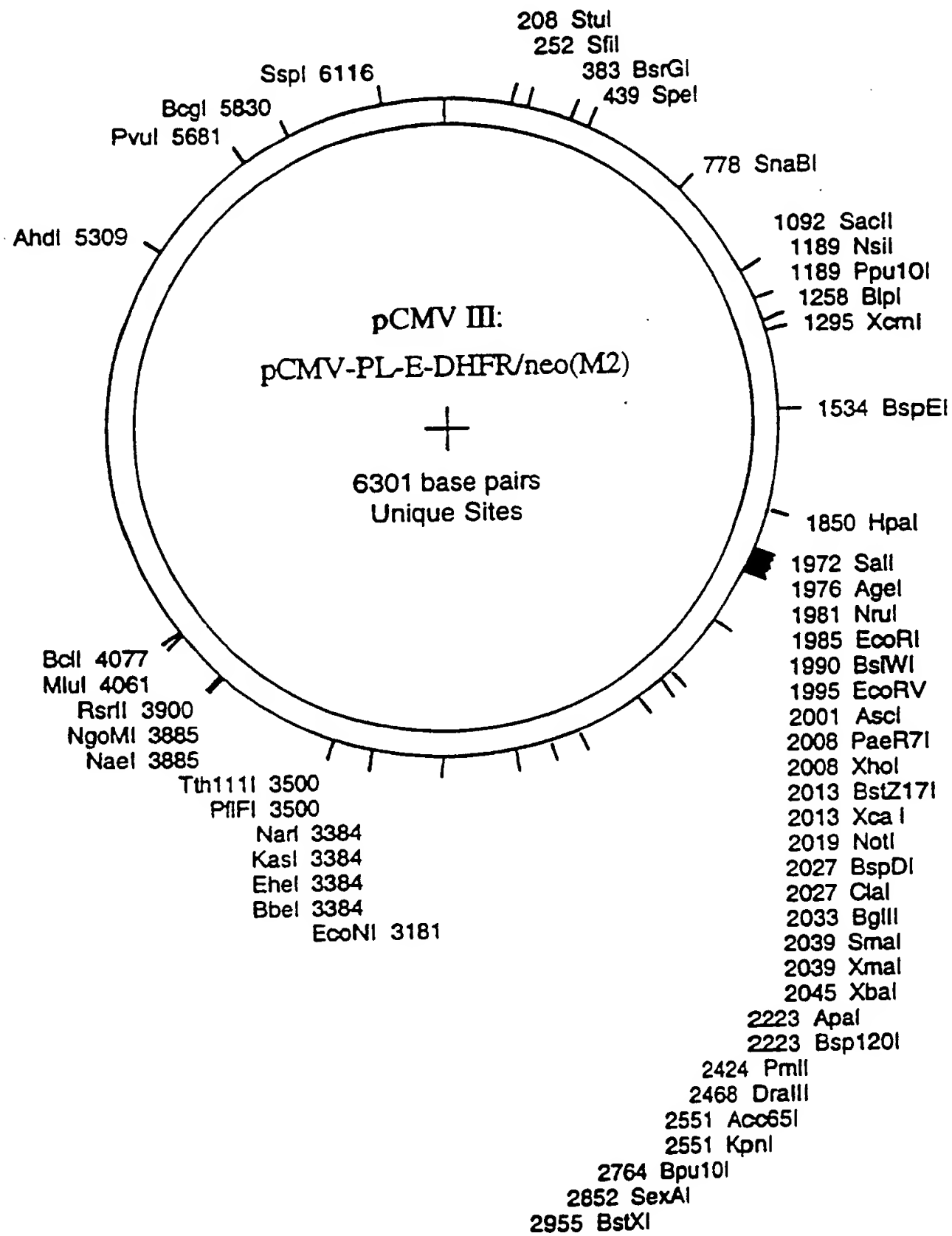


FIG. 13B

SUBSTITUTE SHEET (RULE 26)

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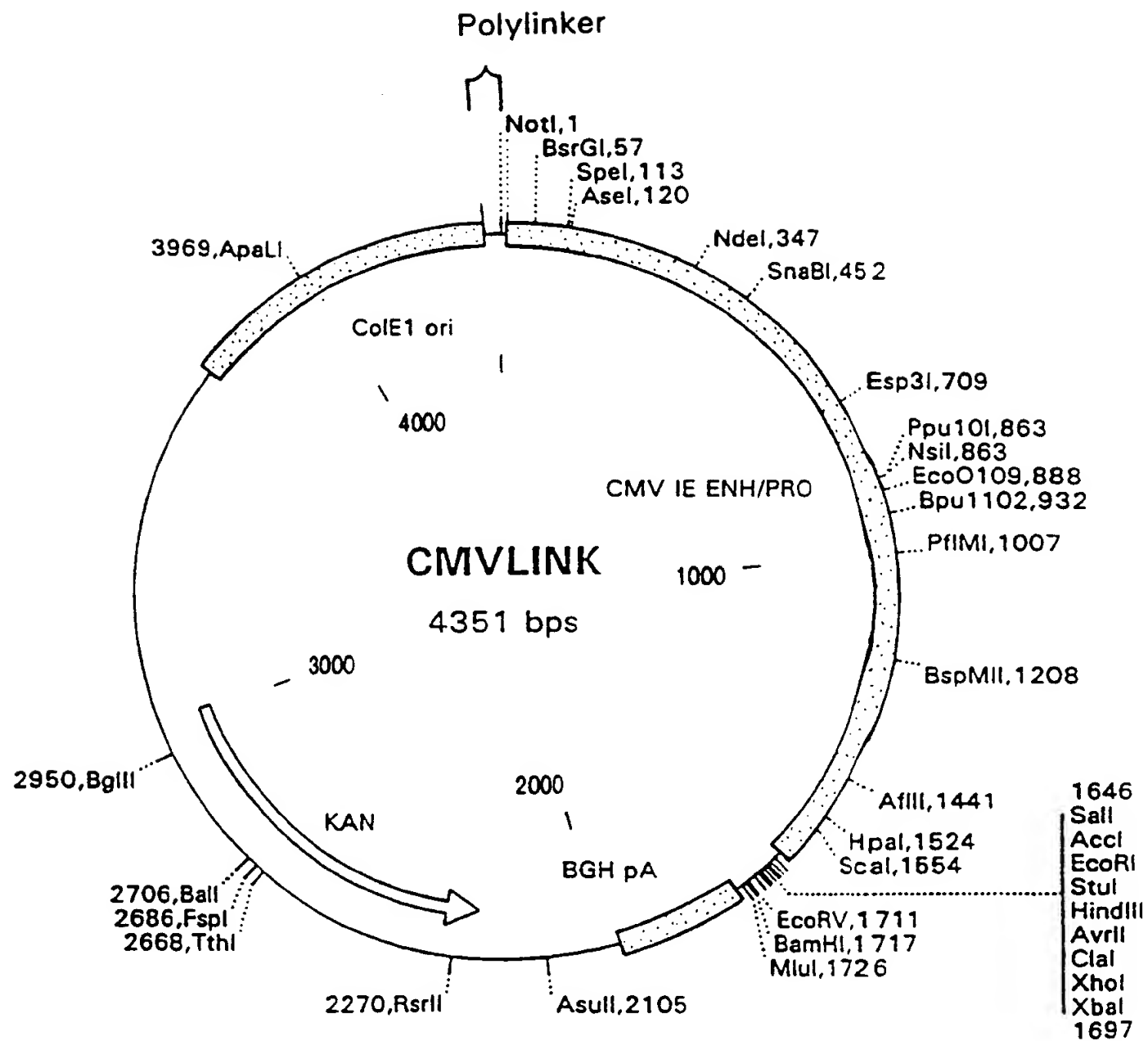


FIG. 14

SUBSTITUTE SHEET (RULE 26)

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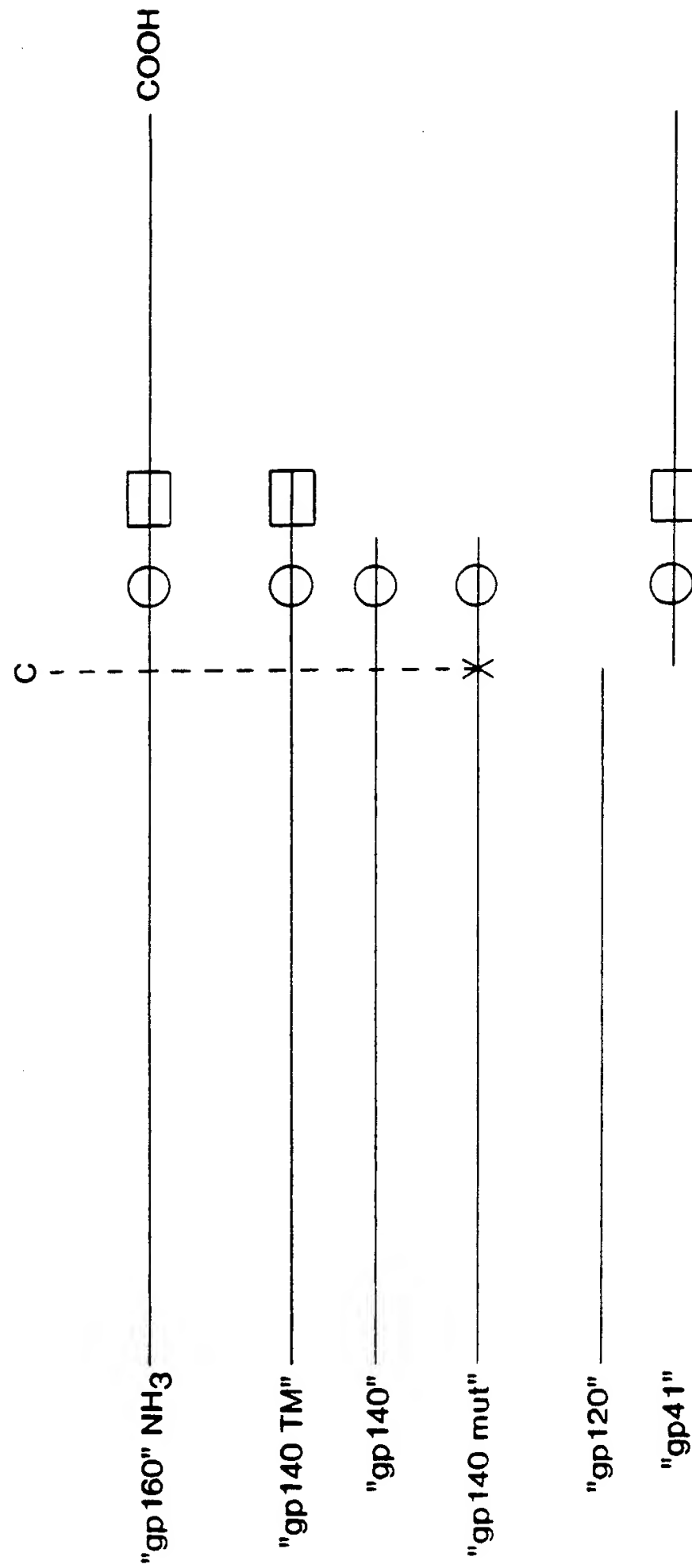


FIG. 15

SUBSTITUTE SHEET (RULE 26)

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gp120wtSF162

GTAGAAAAATTGTGGTCACAGTCTATTATGGGGTACCTGTGTGGAAGAAGCAACCACTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAAATGTCTGGGCCACACATGCCCTGTGTACCCAC
AGACCCCTAACCCACAAGAAATAGTATTGGAAATGTGACAGAAATTTTAAACATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAGTCTAAAGCCATGTGTAAAGTTAACCC
CACTCTGTGTACTCTACATTGCACTAATTTGAAGAAATGCTACTAATACCAAGAGTAGTAATTGGAAAGA
GATGGACAGAGGAGAAATAAAAAATTGCTCTTTCAAGGTCACCACAAGCATAAGAAATAAGATGCAGAAA
GAATATGCACCTTTTATATAAACTTGTAGTAGTACCAATAGATAATAGATAATACAAGCTATAAATTGATAA
ATTGTAACACCTCAGTCATTACACAGGCCCTGTCCAAAGGTATCCTTTGAACCAATTCCCATACATTATTG
TGCCCGGCTGGTTTTCGATTCTAAAGTGTAATGATAAGAAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAAATTAGGCCAGTAGTGTCAACTCAATTGCTGTTAAATGGCAGTC
TAGCAGAAAGAGGGGTAGTAATTAGATCTGAAATTTACAGACAAATGCTAAACTATAATAGTACAGCT
GAAGGAATCTGTAGAAATTAAATTGTACAAGACCCTAACAAATAATACAAGAAAGTATAACTATAGGACCG
GGGAGAGCATTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAAGCACAATTGGGAATAAAACAATAGT
AAAAATGGAAATAACACTTTAAACAGATAGTTACAAAATTACAAGCACAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGACCCAGAAATGTAAATGCACAGTTTAAATTGTGGAGGGAAATTTTTC
TACTGTAAATTCAACACAGCTTTTAAATAGTACTTGGAAATAACTATAGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAAATAAAACAAATTTATAACAGGTGGCAGGAAGTAGGAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAATATTACAGGACTGCTATTAAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCAACCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAAATTGGAGAAAGTG
AATTATATAAATATAAAGTAGTAAAAAATTGAGCCATTAGGAGTAGCACCCCAAGGCAAGAGAAAGAGT
GGTCAGAGAGAAAAAGA

FIG. 16

(SEQ ID NO:30)

SUBSTITUTE SHEET (RULE 26)

gp140wtSF162

GTAGAAAAATTGTGGTCACAGTCTATTATGGGTACCTGTGTGGAAGAAGCAACCACCTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGTCTGGGCCACACATGCCCTGTATCCAC
AGACCCTAACCCACAAGAAATAGTATTGGAAAATGTGACAGAAAATTTTAACATGTGGAATAAATACATG
GTAGAACAGATGCATGAGGATATAATCAAGTTTATGGGATCAAGTCTAAAGCCATGTGTAAGTTAAACCC
CACTCTGTGTACTCTACATTGCACATAATTGCAAGAAATGCTACTAATACCAAGAGTAGTAAATTGGAAAAGA
GATGGACAGAGGAGAAATAAAAATTGCTCTTTCAAGGTCAACCAAGCATAAAGAAATAAGATGCCAGAAA
GAATATGCACCTTTTATATAAATTTGATGTAGTACCAATAGATAATGATAATACAAAGCTATAAATTGATAA
ATTGTAAACACCTCAGTCAATTACACAGGCCCTGTCCAAAGGTATCCTTTGAAACCAATTCCTCATACATTATG
TGCCCCGGCTGTTTGGGATTCTAAAGTGTAAATGATAAGAAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAATTGCTGTTAAATGGCAGTC
TAGCAGAAAGAGGGTAGTAAATTAGATCTGAAAATTTTCACAGACAATGCTAAAACCTATAATAGTACAGCT
GAAGGAAATCTGTAGAAATTAATTGTACAAGACCTAACAAATAATACAAAGAAAGTATAACTATAGGACCG
GGGAGAGCATTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACAAATTGTAACATTAGTGGAG
AAAAATGGAATAACACTTTAAAACAGATAGTTACAAAATTACAAAGCACAAATTTGGGAAATAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGACCCAGAAATTGTAATGCACAGTTTAAATTGTGGAGGGAAATTTTTC
TACTGTAAATTCACACAGCTTTTAAATAGTACTTGGAAATACTATAGGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAAATAAACAATAAATAACAGGTGGCAGGAAGTAGGAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAATAATTACAGGACTGCTATTAAACAAGAGATGGTGGT
AAAGAGATCAGTAACACACCCAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAAATTGGAGAAAGTG
AATTAATAAATAAAGTAGTAAATAATTGAGCCATTAGGAGTAGCACCCACCAAGGCAAGAGAGAGT
GGTGACAGAGAGAAAAGAGCAGTACCGTACCGTACAGCCAGACAAATTAATTGCTGTGGAGCAGGAAAGC
ACTATGGGCGCACGGTCACTGACCGTACCGGTACAGCCAGACAAATTAATTGCTGTAGTGCAACAGC
AGAACAAATTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGCAATCAAGCA
GCTCCAGGCAAGAGTCTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGC
TCTGGAAAACCTCATTTGCACCACTGCTGTGCTTGGAAATGCTAGTTGGAGTAATAAATCTCTGGATCAGA
TTTGGAAATAACATGACCTGGATGGAGTGGGAGAGAGAAAATTGACAAATTACAAAACCTTAATATACACCTT
AATTGAAGAAATCGCAGAACCAAGAAAAGAAATTAATTAGAAATTGGATAAGTGGGCAAGT
TTGTGGAATTGGTTTGACATATCAAAATGGCTGTGGTATATA

FIG. 17

(SEQ ID NO:31)

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gp160wtSF162

GTAGAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGTCTGGGCCACACATGCCTGTGTACCCAC
AGACCCTAACCACAAGAAATAGTATTGGAAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGTCTAAAGCCATGTGTAAAGTTAACCC
CACTCTGTGTTACTCTACATTGCCTAATTTGAAGAATGCTACTAATAACCAAGAGTAGTAATTGGAAAGA
GATGGACAGAGGAGAAATAAAAAATTGCTCTTTCAAGGTCACCACAAGCATAAGAAATAAGATGCAGAAA
GAATATGCACTTTTTTATAAACTTGATGTAGTACCAATAGATAATGATAATACAAGCTATAAATTGATAA
ATTGTAAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAACCAATTCCCATACATTATTG
TGCCCCGGCTGGTTTTGCGATTCTAAAGTGTAATGATAAGAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAATTGCTGTTAAATGGCAGTC
TAGCAGAAGAAGGGGTAGTAATTAGATCTGAAAATTTACAGACAATGCTAAACTATAATAGTACAGCT
GAAGGAATCTGTAGAAATTAATTGTACAAGACCTAACAAATAATACAAGAAAAAGTATAACTATAGGACCG
GGGAGAGCATTTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACATTGTAAACATTAGTGGAG
AAAAATGGAATAACACTTTTAAACAGATAGTTACAAAATTACAAGCACAAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGGACCCAGAAATTGTAATGCACAGTTTTTAATTGTGGAGGGGAATTTTTTC
TACTGTAATTCAACACAGCTTTTTTAATAGTACTTGGAAATAACTATAGGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGGAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAATATTACAGGACTGCTATTAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCACCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAATTGGAGAAGTG
AATTATATAAATATAAAGTAGTAAAAATTGAGCCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGT
GGTGCAGAGAGAAAAAAGAGCAGTGACGCTAGGAGCTATGTTCTTGGGTTCTTGGGAGCAGCAGGAAGC
ACTATGGGCGCACGGTCACTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAGC
AGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCA
GCTCCAGGCAAGAGTCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGC
TCTGGAAAACCTCATTTGCACCACTGCTGTGCCTTGGAAATGCTAGTTGGAGTAATAAATCTCTGGATCAGA
TTTGGAAATAACATGACCTGGATGGAGTGGGAGAGAGAAATTGACAATTACACAACTTAATATACACCTT
AATTGAAGAATCGCAGAACCAACAAGAAAAGAATGAACAAGAATTATTAGAATTGGATAAGTGGGCAAGT
TTGTGGAATTGGTTTGACATATCAAAATGGCTGTGGTATATAAAAATATTCATAATGATAGTAGGAGGTT
TAGTAGGTTTAAGGATAGTTTTTACTGTGCTTTCTATAGTGAATAGAGTTAGGCAGGGATACTCACCATT
ATCATTTTCAGACCCGCTTCCCAGCCCCAAGGGGACCCGACAGGCCCCGAAGGAATCGAAGAAGAAGGTGGA
GAGAGAGACAGAGACAGATCCAGTCCATTAGTGCATGGATTATTAGCACTCATCTGGGACGATCTACGGA
GCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTAATCTTGATTGCAGCGAGGATTGTGGAACCTTCT
GGGACGCAGGGGGTGGGAAGCCCTCAAGTATTGGGGGAATCTCCTGCAGTATTGGATTTCAGGAACATAAG
AATAGTGCTGTAGTTTGTGTTGATGCCATAGCTATAGCAGTAGCTGAGGGGACAGATAGGATTATAGAAG
TAGCACAAAGAATTGGTAGAGCTTTTCTCCACATACCTAGAAGAATAAGACAGGGCTTTGAAAGGGCTTT
GCTATAA

FIG. 18

(SEQ ID NO:32)

SUBSTITUTE SHEET (RULE 26)

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gp140.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgcctggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggcaccaccacctgttctgcccagcgcaccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtggggcgccggcaagctgatcaactgcaacaccagcgtgatcaccagggcctgcccc
aaggtgagccttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagtcaacggcagcggccccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccctggtgagcaccagctgctgctgaacggcagcctggccgaggagggcgctggtgatc
cgcagcagagaacttcaccgacaacgcccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacaccgcgaagagcatcaccatcgggccccggccgcgccttctac
gccaccggcgacatcatcggcgacatccgcccaggccccactgcaacatcagcggcgagaagtggaaac
aacaccctgaagcagatcgtgaccaagctgcaggccccagttcggcaacaagaccatcgtgttcaag
cagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggcgggcgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgggcccccaacaacaccaacggc
accatcaccctgcccctgcccgcacatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgcccccccccatccgcccagatccgctgcagcagcaacatcaccggcctgctgctgaccgc
gacggcgggcaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgcgagcgtgtacaagtacaaggtggtgaagatcgagccctggggcgtggccccccacc
aaggccaagcgccgctggtgcagcgcgagaagcgcgcctgacccctggggcgccatgttccctgggc
ttccctggggcgccgcccgcagcaccatggggcgcccgcagcctgacccctgaccgtgcaggccccgcag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggccccgcgtgctggccgtggagcgctacctg
aaggaccagcagctgctggggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtggggcagcctctggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 24

(SEQ ID NO:37)

SUBSTITUTE SHEET (RULE 26)

gp140.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagaggggtctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctgaagctgacccccctgtgctgggcccggcaactgccagacc
agcgtgatcaccagggcctgccccaaagggtgagcttcgagcccatcccatccactactgcgcccc
gccggcttcgccatcctgaagtgcacgacaagaagttcaacggcagcggccccctgcaccaacgtg
agcaccgtgcagtgacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggaggcggtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacaccccgcaagagcatcacc
atcgcccccgccgcgcttctacgccaccggcgacatcatcggcgacatccgcccaggcccactgc
aacatcagcggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcggcgccgacccccgagatcgtgatgcacagcttc
aactgcggcgggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcacctgccccctgcccgcacagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgcccccccccatccgcccagatccgctgcagcagcaac
atcaccggcctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttcgc
cccggcgggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaaggtggtgaagatc
gagccccctgggctggccccccaccaaggccaagcgccgctggtgcagcgcgagaagcgcgccgtg
accctgggcgccatgttctgggcttctgggcgccgcccagcaccatgggcccgcgagcctg
accctgaccgtgcaggcccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgc
gtgctggcctggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccc
agcctgtggaactgggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 25
(SEQ ID NO:38)

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the pamphlet!

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gp140.mut.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcgcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccctgttctacaagctg
gacgtgggtgcccacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accagggcctgcccgaaggtgagcttcgagcccatcccatccactactgcgcccccgccggcttc
gccatcctgaagtgcaacgacaagaagtccaacggcagcggccccctgcaccaacgtgagcaccgtg
cagtgcacccacggcatccgccccgtgggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcgtgggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacaccccgcaagagcatcaccatcggcccc
ggccgcgcccttctacgccaccggcgacatcatcgccgacatccgccaggccccactgcaacatcagc
ggcgagaagtggacaacacccctgaagcagatcgtgaccaagctgcaggccccagttcgggaacaag
accatcgtgttcaagcagagcagcggcgccgagaccccgagatcgtgatgcacagcttcaactgcggc
ggcgagtcttcttactgcaacagcaccagctgttcaacagcacctggaacaacaccatcggcccc
aacaacaccaacggcaccatcaccctgccctgcccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcccgcagatccgctgcagcagcaacatcaccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccagatcttcgccccggcggc
ggcgacatgcgcgacaactggcgagcgagctgtacaagtacaagggtggatgaagatcgagccccctg
ggcgtggccccccaccaaggccaagcgcccgctgggtgcagcgcgagaagagcgccgtgacctgggc
gccatgttcctgggcttcctgggcgccggccggcagcaccatgggcgcccgagcctgacctgacc
gtgcaggccccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgctacctgaaggaccagcagctgctggggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgccctggaacgccagctggagcaacaagacccctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactgggttcgacatcagcaagtggctgtggtacatctaaactcgag

FIG. 26

(SEQ ID NO:39)

gp140.mut.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccacccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcgcgagatcaagaactgc
agcttcaaggtggggcgccggcaagctgatcaactgcaacaccagcgtgatcaccagggcctgcccc
aaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcgggccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggaggcggtggtgatc
cgagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacacccgcaagagcatcaccatcgcccccgccgcgccttctac
gccaccggcgacatcatcgggcgacatccgcccaggcccactgcaacatcagcggcgagaagtggaa
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggaacaagaccatcgtgttcaag
cagagcagcgggcgccgaccccgagatcgtgatgcacagcttcaactgcggcgggcgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacacccatcgcccccaacaacaccaacggc
accatcaccttgccttgcgcgatcaagcagatcatcaaccgctggcaggagggtgggcaaggccatg
tacgccccccccatccgcgggccagatccgctgcagcagcaacatcacccggcctgctgctgacccgc
gacggcggaaggagatcagcaacaccaccagatcttccgccccggcgggcgacatgcgcgac
aactggcgcgagcagctgtacaagtacaaggtggtgaagatcgagccccctggcggtggccccacc
aaggccaagcgccgctggtgcagcgcgagaagagcgccgtgaccctgggcgccatgttcttgggc
ttcttggcgcccgccggcagcaccatggggcgcccgagcctgaccctgacgctgcaggccccgag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggcccgcgtgctggcgtggagcgctacctg
aaggaccagcagctgctggggcatctggggctgcagcggaagctgatctgcaccaccgcccgtgcc
tggaacgcccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 27

(SEQ ID NO:40)

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gp140.mut.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagaggggctctgctgtgtgctgctgctgtgtggagcagtc
ttcggtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggtgggcccggcaactgccagacc
agcgtgatcaccacaggcctgccccaaagggtgagcttcgagcccatcccatccactactgcgcccc
gcccgttctgccatcctgaagtgcacgacaagaagtccaacggcagcggccccctgcaccaacgtg
agcaccgtgcagtgacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggaggcggtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcacc
atcgggccccggccgccttctacgccaccggcgacatcatcgggcgacatccggcaggccccactgc
aacatcagcggcgagaagtggaaacaacaccctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttc
aactgcggcgggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgggcccccaacaacaccaacggcaccatcacccctgccccgcccgcacaaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgcccccccccatccgcgggccagatccgctgcagcagcaac
atcacccggcctgctgctgacccgcgacggcgggcaaggagatcagcaacaccaccgagatcttccgc
ccccggcgggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaagggtggtgaagatc
gagccccctggcgctggccccccaccaaggccaagcgccgctggtgcagcgcgagaagagcgccgtg
accctggggcgccatgttccctgggcttccctggggcgcccgccggcagcaccatggggcgcccgagcctg
accctgaccgtgcaggccccgcccagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctggggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 28

(SEQ ID NO:41)

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gp140.mut7.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccacccacgcctgcgtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcgtgaagctgacccccctgtgcgtgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcctgttctacaagctg
gacgtgggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accaggcctgccccaaaggtagcttcgagcccatccccatccactactgcgcccccgccggcttc
gccatcctgaagtgcacgacaagaagtcaacggcagcggccccctgcaccaacgtgagcaccgtg
cagtgcaccacggcatccgccccgtggtagcaccacagctgctgctgaacggcagcctggccgag
gagggcggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacaacaccgcgaagagcatcaccatcgcccc
ggccgcgccttctacgccaccggcgacatcatcggcgacatccgccaggccccactgcaacatcagc
ggcgagaagtggaacaacacctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaag
accatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcaccacagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcaccctgccctgccgcacatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaacatcaccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgccagcagctgtacaagtacaagggtggtgaagatcgagccccctg
ggcgtggccccccaccaaggccatcagcagcgtgggtgcagagcgagaagagcgccgtgaccctgggc
gccatgttccctgggcttctgggcgcccggcgagcaccatgggcgccccgagcctgaccctgacc
gtgcaggccccgcccagctgctgagcggcatcgtgcagcagcagaacaacctgctgogcgccatcgag
gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactgggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 29

(SEQ ID NO:42)

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gp140.mut7.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccacccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcgcgagatcaagaactgc
agcttcaagggtggcgccggcaagctgatcaactgcaacaccagcgtgatcaccagggcctgcccc
aaggtagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagtcaacggcgagcggccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcgagcctggccgaggaggcggtggtgatc
cgagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcaccgcccccaacaacacccgcaagagcatcaccatcgcccccgccgcgccttctac
gccaccggcgacatcatcgccgacatccgccaggccccactgcaacatcagcggcgagaagtggaac
aacaccctgaagcagatcgtgaccaagctgcaggccccagttcggaacaagaccatcgtgttcaag
cagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggcgggcgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacacccatcgcccccaacaacaccaacggc
accatcacccctgccctgccgcacatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgcccccccccatccgcgccagatccgctgcagcagcaacatcacccggcctgctgctgacccgc
gacggcggaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgagcgagctgtacaagtacaagggtggtgaagatcgagccctgggctggccccccacc
aaggccatcagcagcgtggtgcagagcgagaagagcgccgtgacccctgggcgccatgttcctgggc
ttcctgggcgcccgccggcagcaccatgggcgccccgagcctgacccctgacccgtgcaggccccgcccag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggccgtggagcgctacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacaccctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 30

(SEQ ID NO:43)

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gp140.mut7.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggcaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgggcccggcaactgccagacc
agcgtgatcaccagggcctgcccgaagggtgagcttcgagcccatcccatccactactgcccctcc
gccggcttcgccatcctgaagtgcacacgaagaagtccaacggcagcggccccctgcaccaacgtg
agcaccgtgcagtgacccacggcatccgccccgtgggtgagcaccagctgctgctgaacggcagc
ctggccgaggaggcggtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcacc
atcgggccccggcgcgcttctacgccaccggcgacatcatcggcgacatccgcccaggcccactgc
aacatcagcggcgagaagtggacaacacccctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcggcgggcgaccccgagatcgtgatgcacagcttc
aactgcggcgggcagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgggccccaacaacaccaacggcaccatcaccctgccctgccgcatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgccccccccatccgcccagatccgctgcagcagcaac
atcaccggcctgctgctgacccgcgacggcgggcaaggagatcagcaacaccaccgagatcttccgc
ccccggcgggcgacatgcgcgacaactggcgagcagctgtacaagtacaagggtggtgaagatc
gagccccctggcgctggccccccaccaaggccatcagcagcgtggtgcagagcgagaagagcgcctg
accctggggcgccatgttccctgggcttccctggggcgcccgccggcagcaccatggggcgcccgagcctg
accctgaccgtgcaggcccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctggggcatctggggctgcagcggcaag
ctgatctgcaccacgcgcctgcccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccc
agcctgtggaactgggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 31

(SEQ ID NO:44)

gp140.mut8.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacctgcactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcgcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccctgttctacaagctg
gacgtgggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accagggcctgcccccaaggtgagcttcgagcccatcccatccactactgcgcccccgccggcttc
gccatcctgaagtgcacgacaagaagtccaacggcagcgcccccgcaccaacgtgagcacccgtg
cagtgcacccacggcatccgccccgtgggtgagcacccagctgctgctgaacggcagcctggccgag
gagggcgctggatccgagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcaccatcgcccc
ggccgcgccttctacgcccaccggcgacatcatcgggcgacatccgccaaggcccaactgcaacatcagc
ggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggcccagttcggaacaag
accatcgtgttcaagcagagcagcggcgggcgaccccgagatcgtgatgcacagcttcaactgcggc
ggcgagtctcttctactgcaacagcacccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcacctgcccgtgcccgcacatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcgccagatccgctgcagcagcaacatcacccgc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcagctgtacaagtacaaggctggtgaagatcgagcccctg
ggcggtggccccaccatcgccatcagcagcgtggtgcagagcgagaagagcgccgtgacctgggc
gccatgttccctgggcttccctggcgcccgccggcagcaccatggggcgcccgagcctgacctgacc
gtgcaggcccccgagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gcccagcagcacctgctgcagctgacctgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgtacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgcccgggaacgcccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgaggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 32

(SEQ ID NO:45)

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gp140.mut8.modSF162.delV2

gaattcgccaccatggatgcaatgaagagaggggtctgctgtgtgctgctgctgtgtggagcagtc
ttcggtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccacccacgcctgctgccccaccgacccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcgccgagatcaagaactgc
agcttcaagggtggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aagggtgagcttcgagcccatccccatccactactgcgccccccgcccgttcgccatcctgaagtgc
aacgacaagaagtccaacggcagcggccccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggaggggcgtggtgatc
cgagcgagaaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacaccgcgaagagcatcaccatcgcccccgccgcgccttctac
gccaccggcgacatcatcgccgacatccgcccaggcccactgcaacatcagcggcgagaagtggaa
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggaacaagaccatcgtgttcaag
cagagcagcggcgccgaccccgagatcgtgatgcacagcttcaactgcggcgggcagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccccaacaacaccaacggc
accatcacctgcccgtccgcacatcaagcagatcatcaaccgctggcaggagggtgggcaaggccatg
tacgccccccccatccgcccagatccgctgcagcagcaacatcacccgctgctgctgacccgc
gacggcggaaggagatcagcaacaccaccgagatcttccgcccccgccggcgacatgcgcgac
aactggcgagcagcgtgtacaagtacaagggtggtgaagatcgagccctgggctggccccacc
atcgccatcagcagcgtggtgcagagcgagaagagcgccgtgacccctgggcgccatgttccctgggc
ttcctgggcgccgcccggcagcaccatgggcccgcagcctgacccctgaccgtgcaggccccgcccag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggccgtggagcgctacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtggcccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 33

(SEQ ID NO:46)

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gp140.mut8.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggccaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgcggtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcggtgaagctgacccccctgtgctggggcgccggcaactgccagacc
agcgtgatcaccagggcctgcccccaagggtgagcttcgagcccatcccatccactactgcgcccc
gccggcttcgccatcctgaagtgcacgacaagaagtccaacggcagcgggccctgcaccaacgtg
agcaccgtgcagtgacccacggcatccgccccgtgggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcggtgatccgcagcgagaacttcaccgacaacgcgaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcacc
atcgggccccggccgcgccttctacgccaccggcgacatcatcgggcagatccgccaggccactgc
aacatcagcgggcgagaagtggaaacaacaccctgaagcagatcgtgaccaagctgcaggccagttc
ggcaacaagaccatcgtgttcaagcagagcagcgggcgaccccgagatcgtgatgcacagcttc
aactgcggcgggcaggttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgggcccaacaacaccaacggcaccatcacctgcccctgcccgcacagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgcccccccccatccgcgggccagatccgctgcagcagcaac
atcacccggcctgctgctgacccgcgacggcgggcaaggagatcagcaacaccaccgagatctccgc
ccccggcgggcgacatgcgcgacaactggcgagcagcgtgtacaagtacaagggtgggtgaagatc
gagccccctgggctggggccccaccatcgccatcagcagcgtgggtgcagagcgagaagagcgccgtg
accctggggcgccatgttccctgggcttccctggggcgcccgccggcagcaccatggggcgcccgagcctg
accctgaccgtgcaggccccgagctgctgagcgccatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccg
gtgctggccgtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgccctggaaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccc
agcctgtggaactggttcgacatcagcaagtggctgtgtgtacatctaactcgag

FIG. 34

(SEQ ID NO:47)

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gp160.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgcctggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgcgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accaggcctgcccccaaggtgagcttcgagcccatccccatccactactgcgcccccgccggttc
gccatcctgaagtgcacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcacctg
cagtgcacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcgtggtgatccgcagcagagaacttcaccgacaacgccaaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacaccccgcaagagcatcaccatcgcccc
ggccgcgccttctacgccaccggcgacatcatcggcgacatccgcccaggcccactgcaacatcagc
ggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaag
accatcgtgttcaagcagagcagcggcgggcgaccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcacctgcccctgccgcacatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaacatcacccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcagctgtacaagtacaaggtggtgaagatcgagccccctg
ggcgtggcccccaaccaaggccaagcgccgcgtggtgcagcgcgagaagcgcgccgtgacctgggc
gccatgttctctgggcttctgggcgcgcgcggcagcaccatgggcgcgcgcagcctgacctgacc
gtgcaggccccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgcctatcgag
gcccagcagcacctgctgcagctgacctgtggggcatcaagcagctgcaggccccgcgtgctggcc
gtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatcaagatcttcatcatgatcgtgggcggcctg
gtgggcctgcgcacatcgtgttaccgtgctgagcatcgtgaaccgcgtgcgcccagggtacagcccc
ctgagcttccagaccgccttccccgccccccgcggcccccgaccgccccgagggcatcgaggaggag
ggcggcgagcgcgacccgcgaccgcagcagccccctggtgcaaggcctgctggccctgatctgggac
gacctgcgcagcctgtgctgttcagctaccaccgcccgcgcgacctgatcctgatcgccgccccgc
atcgtggagctgctgggcgcgcgcggctgggaggccctgaagtaactggggcaacctgctgcagtac
tggatccaggagctgaagaacagcgcctgagcctgttcgacgccatcgccatcgccgtggccgag
ggcaccgaccgcatcatcgaggtggcccagcgcacggccgccttctgcacatcccccgccgc
atccgcccagggttcgagcgcgccttctgtgaactcgag

FIG. 35

(SEQ ID NO:48)

gp160.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggcaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctgaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcccgcagatcaagaactgc
agcttcaaggtgggcgcccggcaagctgatcaactgcaacaccagcgtgatcaccagggcctgcccc
aaggtgagcttcgagcccatcccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagtcaacggcagcgccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggaggcggtggtgatc
cgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcaccgcgcccaacaacaacaccgcgaagagcatcaccatcgcccccggccgcgccttctac
gccaccggcgacatcatcggcgacatccgccaggcccactgcaacatcagcggcgagaagtggaaac
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggaacaagaccatcgtgttcaag
cagagcagcggcgccgaccccgagatcgtgatgcacagcttcaactgcggcgggcagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccccacaacaaccaacggc
accatcaccttgccttgcgcacatcaagcagatcatcaacccgctggcaggagggtgggcaaggccatg
tacgcccccccccatccgcggccagatccgctgcagcagcaacatcacccggcctgctgctgaccgc
gacggcggaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgagcagctgtacaagtacaaggtggtgaagatcgagccctgggctggccccacc
aaggccaagcgccgctggtgcagcgcgagaagcgcgccgtgaccctgggcgccatgttcttgggc
ttcttgggcgcccggcagcaccatgggcgcccgagcctgaccctgaccgtgcaggcccgccag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcccgcacatcgaggcccgagcagcactg
ctgcagctgaccgtgtggggcatcaagcagctgcaggcccgctgctggccgtggagcgtacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactgggttcgacatc
agcaagtggctgtggtacatcaagatcttcatcatgatcgtgggcccctgggtgggcctgcgcac
gtgttcaccgtgctgagcatcgtgaaccgctgcgccagggtacagccccctgagcttccagacc
cgcttccccgcccccgccgccccgacccgccccgagggcatcgaggaggaggcgggcgagcgcgac
cgcgaccgcagcagccccctggtgcacggcctgctggccctgatctgggacgacctgcgcagcctg
tgccctgttcagctaccaccgctgcgcgacctgatcctgatcgccgccccgcatcgtggagctgctg
ggccgcccggctgggaggccctgaagtactggggcaacctgctgcagtactggatccaggagctg
aagaacagcggcgtgagcctgttcgacgcccacgcccacgcccgtggccgagggcaccgaccgcatc
atcgaggtggcccagcgcacatcgccgcccgccttccctgcacatccccgcccgcacccgcccagggttc
gagcgcgcccctgctgtaactcgag

FIG. 36

(SEQ ID NO:49)

gp160.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcggtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggcaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgccccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggggcgccggcaactgccagacc
agcgtgatcaccagggcctgcccccaagggtgagcttcgagcccatcccatccactactgcgcccc
gcccgttcgccatcctgaagtgcacgacaagaagttcaacggcagcggccccctgcaccaacgtg
agcaccgtgcagtgacccacggcatccgccccgtgggtgagcaccagctgctgctgaacggcagc
ctggccgaggaggcggtggtgatccgcagcgagaacttcaccgacaacgc caagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcacc
atcgggccccggcgcgcccttctacgccaccggcgacatcatcggcgacatccgcccaggcccactgc
aacatcagcggcgagaagtgggaacaacaccctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttc
aactgcggcgggcgagttcttctactgcaacagcaccagctgttcaacagcacctgggaacaacacc
atcgggccccaacaacaccaacggcaccatcaccctgcccctgcgcgatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgccccccccatccgcccagatccgctgcagcagcaac
atcaccggcctgctgctgacccgcgacggcgggcaaggagatcagcaacaccaccgagatcttccgc
cccggcgggcgggcgacatgcgcgacaactggcgcgagcagctgtacaagta caaggtgggtgaagatc
gagccccctggcgctggccccccaccaaggcccaagcgccgctggtgcagcgcgagaagcgcgccgtg
accctggggcgccatgttccctgggcttccctggggcgccggcgagcaccatggggcgcccgagcctg
accctgaccgtgcaggccccgagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgc
gtgctggccgtggagcgtacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgcccctggaaacgcccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacaccctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtggggc
agcctgtggaactgggttcgacatcagcaagtggctgtggtacatcaagatcttcatcatgatcgtg
ggcgccctggtgggcttgcgcacgtgttccaccgtgctgagcatcgtgaaccgcgtgcgccagggc
tacagccccctgagcttccagacccttccccgcccccgcgccccgaaccgccccgagggcac
gaggaggagggcgggcgagcgcgaccgcgaccgcagcagccccctgggtgcacggcctgctggccctg
atctgggacgacctgcgcagcctgtgcctgttcagctaccaccgacctgcgcgaacctgatcctgatc
gccgccccgcatcgtggagctgctgggccccgcccggctgggaggccctgaagtactggggcaacctg
ctgcagtactggatccaggagctgaagaacagcgccgtgagcctgttcgacgcccacatcgccatcgcc
gtggccgagggcaccgaccgcatcatcgaggtggccagcgcacatcggcgcgccttccctgcacatc
ccccgcccgcacccgcccagggttcgagcgcgccctgctgtaactcgag

FIG. 37

(SEQ ID NO:50)

49 / 131

gp120wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACA
TGGTGGAAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTACTGATAAGT
TGACAGGTAGTACTAATGGCACAAATAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATACATTATTGTGCCCCGG
CTGGTTTTGCGATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAATACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTAATTG
TGGAGGGGAATTTTTCTATTGTAATACATCACAACCTATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAGGG
CTGCTATTAAGTAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAGAAAAGA

FIG. 38

(SEQ ID NO:51)

50 / 131

gp140wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACA
TGGTGGAAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTACTGATAAGT
TGACAGGTAGTACTAATGGCACAAATAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATACATTATTGTGCCCCGG
CTGGTTTTTGCGATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAATACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTATTTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTTAATTG
TGGAGGGGAATTTTTCTATTGTAAATACATCACAATAATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCTCCCATCAGAGGACAAATTAATGTTCATCAAATATTACAGGG
CTGCTATTAAGTAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAAAAGAGCAGTGGGACTAGGAG
CTTTGTTCAATTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTC
AGTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAG
CAGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA
CGGTCTGGGGCATCAAACAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA
CCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGCTCTGGAAAACCTCATTT
GCACCACTACTGTGCCTTGGAACCTAGTTGGAGTAATAAATCTCTGACTGAG
ATTTGGGATAATATGACCTGGATGGAGTGGGAAAGAGAAATTGGCAATTATA
CAGGCTTAATATACAATTTAATTGAAATAGCACAAAACCAGCAAGAAAAGAA
TGAACAAGAATTATTGGAATTAGACAAGTGGGCAAGTTTGTGGAATTGGTTT
GATATAACAACTGGCTGTGGTATATA

FIG. 39
(SEQ ID NO:52)

gp160wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACA
TGGTGGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTACTGATAAGT
TGACAGGTAGTACTAATGGCACAATAAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATACATTATTGTGCCCCGG
CTGGTTTTTGCATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAATACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTTTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTTAATTG
TGGAGGGGAATTTTTCTATTGTAAATACATCACAACCTATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAGGG
CTGCTATTAAGTAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAGAAAAGAGCAGTGGGACTAGGAG
CTTTGTTTCATTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTC
AGTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAG
CAGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA
CGGTCTGGGGCATCAAACAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA
CCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGCTCTGGAAAACCTCATT
GCACCACTACTGTGCCTTGGAACCTAGTTGGAGTAATAAATCTCTGACTGAG
ATTTGGGATAATATGACCTGGATGGAGTGGGAAAGAGAAATTGGCAATTATA
CAGGCTTAATATACAATTTAATTGAAATAGCACAAAACAGCAAGAAAAGAA
TGAACAAGAATTATTGGAATTAGACAAGTGGGCAAGTTTGTGGAATTGGTTT
GATATAACAACTGGCTGTGGTATATAAGAATATTCATAATGATAGTAGGAG
GCTTGATAGGTTTAAGAATAGTTTTTGTCTGTACTTTCTATAGTGAATAGAGTT
AGGCAGGGATACTACCAATATCATTGCAGACCCGCCTCCCAGCTCAGAGGG

FIG. 40A

(SEQ ID NO:53)

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GACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGTGGAGAGAGAGACAGA
GACAGATCCAATCGATTAGTGCATGGATTATTGGCACTCATCTGGGACGATCT
GCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTG
TAGCGAGGATTGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAGTA
TTGGTGGAATCTCCTGCAGTATTGGAGTCAGGAGCTAAAGAGTAGTGCTGTT
AGTTTGTTTAATGCCACAGCAATAGCAGTAGCTGAAGGGACAGATAGGATTA
TAGAAATAGTACAAAGAATTTTATAGAGCTGTAATTCACATACCTAGAAGAAT
AAGACAGGGCTTGGAGAGGGCTTTACTATAA

FIG. 40B
(SEQ ID NO:53)

gp120.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGGTGCCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACCGACAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAAGTGCAGCTTCAACATCACCAACAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCCCTGCAAGAACGTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGACGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAGAAAGCTGCGCGAGCAGTTCCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCCACCCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGCGCTAAGATATCGGATCCTCTAGA

FIG. 41
(SEQ ID NO:54)

gp120.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACCGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGCGGGGAGGGAAGTGCAGAGACCAGCGTGATCACCCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCCGGCCCCCTGCAAGAACGTGAGC
ACCGTGCAAGTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGA
CCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCCGGCCGCGCCTTCTACGCCACCCGGCGACATCAT
CGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCG
AGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGGCCCCCACCAGGC
CAAGCGCCGCGTGGTGCAGCGCGAGAAGCGCTAAGATATCGGATCCTCTAGA

FIG. 42

(SEQ ID NO:55)

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gp140.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCAGCGGCACCAACAGCACCAAGCACCAACAGCACCGACAGCTGGGAGAAAGATG
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGCAACCAACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGACGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCCGGCCGCGCCTTCT
ACGCCAACGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGAGAGAGCTGCGCGAGCAGTTCCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCAATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCGCCCCACCCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAGCGCGCCGTGGGCGCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGAGAACAACCTGCTGCGCGCCATCGAGGCCCGAGCAGCACCTGCTGC
AGCTGACCGTGTTGGGCGATCAAGCAGCTGACGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCGATCTGGGCGTGACGCGGCAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACCCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAAGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 43

(SEQ ID NO:56)

gp140.mut.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTGCCCGTG
TGGAAGGAGGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGGCACCAACAGCACCAGCGGCAC
CAACAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACCAGCAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCGGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGGCCCCTGCAAGAACGTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCCGGCCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAAGAGCTGCGCGAGCAGTTCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCAATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCCCCCACCAGGCCAAGCGCCGC
GTGGTGCAAGCGGAGAAGAGCGCCGTGGGCGCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCCTCCGTGACCCTGACCGTGCAAGGCCCGCCAGCTGCTGAG
CGGCATCGTGCAAGCAGCAGAAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGC
AGCTGACCGTGTTGGGGCATCAAGCAGCTGCAAGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAAGTGGTTCGACATCACCACCTGGCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 44

(SEQ ID NO:57)

gp140.TM.modUS4

GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCCAGCGGCAC
CAACAGCACCCAGCGGCACCAACAGCACCCAGCACCAACAGCACCCAGCAGCTGGGAGAAGATG
CCCGAGGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCCGGCCCCCTGCAAGAACGTGAGCACCGTGCAAGTGCACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTACCGACAACGCCAAGACCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGC
GTGGTGACGCGGAGAAAGCGCGCCGTGGGCGCTGGGCGCCTGTTTCATCGGCTTCCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCCGCGAGCTGCTGAG
CGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGC
AGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCAACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAACTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCTTTCATCATGATCGTGGGCG
GCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGTAAGATATCGGATCCTCTA
GA

FIG. 45

(SEQ ID NO:58)

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Gp140modUS4.DV1V2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGCCACCCA
CGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGGTGAACCTGACCAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCCGGCC
AGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
CGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGC
CCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGGCATCCGCCCCGTGG
TGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCT
GCGCTCCGAGAACTTACCCGACAACGCCAAGACCATCATCGTGCACTGAAC
GAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAGAGCA
TCCACATCGGCCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGA
CATCCGCCAGGCCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTC
GAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATC
ATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCA
ACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCAC
CTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT
CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAG
GCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAAGCAATATTA
CCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAACGA
CACCGAGACCTTCCGCCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCATCGAGCCCCTGGGCGTGGCCCCCA
CCCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGCGCGCCGTGGGCCTGG
GCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCGGGAGCACCATGGGCGCCGC
CTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCTGAGCGGCATCGTGCA
CAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCAGC
TGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCG
CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTG
ATCTGCACCAACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGA
CCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCA
ACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGA
GAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAA
CTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTCTA
GA

FIG. 46

(SEQ ID NO:59)

59 / 131

Gp140modUS4.DV2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGGCCACCACCACCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGGCCACCCA
CGCCTGCGTGCCCAACCGACCCCAACCCCAAGGAGGTGAACCTGACCAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCC
CCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGG
CACCAACAGCACCGAGCGGCACCAACAGCACCGAGCGGCACCAACAGCACCG
CACCAACAGCACCGACAGCTGGGAGAAGATGCCCCAGGGCGAGATCAAGAA
CTGCAGCTTCAACATCGGCGCCCGGCCGCTGATCAACTGCAACACCAGCGTG
ATCACCCAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACT
GCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGG
CACCGGCCCTGCAAGAACGTGAGCACCGTGAGTGACCCACGGCATCCGC
CCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGA
TCGTGCTGCGCTCCGAGAACTTACCGACAACGCCAAGACCATCATCGTGCA
GCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGT
AAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCA
TCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAA
CACCTTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAA
GACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCAC
AGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAA
CAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACAC
CATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTG
GGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGACAGCAGCA
ATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCAC
CAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTG
GCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTG
GCCCCACCCAGGCCAAGCGCCGCGTGGTGACGCGGAGAAGCGCGCCGTG
GGCCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCGGGAGCACCATGG
GCGCCGCCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAGCGGCAT
CGTGACAGCAGCAGAACAACTGCTGCGCGCCATCGAGGCCCGAGCAGCACCTG
CTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCG
TGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGG
CAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAG
AGCCTGACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAG
ATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC
AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCC
TGTGGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGG
ATCCTCTAGA

FIG. 47

(SEQ ID NO:60)

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Gp140modmutUS4.DV1V2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGCCACCC
ACGCCTGCGTGCCCAACGACCCCAACCCCAAGGAGGTGAACCTGACCAACGT
GACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGA
GGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCCGGC
CAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCC
CCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGG
CCCCTGCAAGAACGTGAGCACCGTGCAAGTGCAACCCACGGCATCCGCCCCGTG
GTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGC
TGCGCTCCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAA
CGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAACACGCGTAAGAGC
ATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCG
ACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCT
CGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCAT
CATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCACAGCTTC
AACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCA
CCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCA
TCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAA
GGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAATATT
ACCGGCCTGCTGCTGACCCGCGACGGCGGGCACCAACAACAACCGCACCAACG
ACACCGAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAACCTGGCGCA
GCGAGCTGTACAAGTACAAGGTGGTGCATCGAGCCCCTGGGCGTGGCCCC
CACCCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGAGCGCCGTGGGCCT
GGGCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCCGGGAGCACCATGGGCGCC
GCCTCCGTGACCCTGACCGTGCAAGGCCCGCCAGCTGCTGAGCGGCATCGTGC
AGCAGCAGAACAACTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCA
GCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAG
CGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGC
TGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGG
CAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACCAGCAG
GAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGG
AACTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTC
TAGA

FIG. 48

(SEQ ID NO:61)

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gp140.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACAGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGCGGGGAGGGAACTGCGAGACCAGCGTGATCACCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCCCTGCAAGAACGTGAGC
ACCGTGCAAGTGCAACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGA
CCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCCGGCCGCGCCTTCTACGCCACCGGGCGACATCAT
CGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCG
AGCAGATCGTGGAAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCCGGCCGCGGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCGCCCCACCCAGGC
CAAGCGCCGCGTGCTGCGAGCGCGAGAAGCGCCCGTGCGGCTGGGCGCCCTGTTTCATCG
GCTTCCTGGGCGCCGCGGGGAGCACCATGGGCGCCGCTCCGTGACCCCTGACCGTGCGAG
GCCCCGCGAGCTGCTGAGCGGCATCGTGCGAGCAGCAGAACAACCTGCTGCGCGCCATCGA
GGCCCAGCAGCACCTGCTGCGAGCTGACCGTGTGGGGCATCAAGCAGCTGCGAGGCCCGCA
TCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCGAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACA
CCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAG
GAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTG
GCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 49

(SEQ ID NO:62)

62/131

gp140.mut.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACAGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGGGGGCAGGGAAGTGCAGAGACCAGCGTGATCACCCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACC GGCCCCCTGCAAGAACGTGAGC
ACCGTGCAAGTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGA
CCATCATCGTGCAAGTGCAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCAT
CGGCGACATCCGCCAGGCCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCG
AGCAGATCGTGGAAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCATCGAGCCCCCTGGGCGTGGCCCCCACCAGGC
CAAGCGCCGCGTGGTGCAGCGCGAGAAGAGCGCCGTGGGCCTGGGCGCCCTGTTTCATCG
GCTTCCTGGGCGCCGCGGGAGCACCATGGGCGCCGCTCCGTGACCCCTGACCGTGACG
GCCCGCCAGCTGCTGAGCGGCATCGTGACGAGCAGAACAACCTGCTGCGCGCCATCGA
GGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCA
TCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACA
CCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAG
GAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAAGTGGTTCGACATCACCAGCTG
GCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 50

(SEQ ID NO:63)

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gp160.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTG
TGGAAGGAGGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACCAGCAGCTGGGAGAAGATG
CCCGAGGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCCGGCCCCCTGCAAGAACGTGAGCACCGTGCAAGTGCACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAAGAGCTGCGCGAGCAGTTCCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCCCCCAACCGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGCGCGCCGTGGGCGCCCTGTTTATCGGCTTCTTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGCCCAGCAGCACCTGCTGC
AGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCCGATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCTTCAATCATGATCGTGGGCG
GCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCT
ACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAGCGCGGCCCGACCGCCCCGAGGGC
ATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGCAACCGCCTGGTGCACGGCCTGCT
GGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCTGTTACGCTACCAACCGCCTGCGCGACCT
GCTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCGCGCGGCTGGGAGGCCCTGAAGT
ACTGGTGGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTC
AACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGACGCGCAT
CTTCCGCGCCGTGATCCACATCCCCCGCCGATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTA
AGATATCGGATCCTCTAGA

FIG. 51

(SEQ ID NO:64)

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gp160.modUS4.delV1

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTTCGCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGGGGCGCCGGCGGCGAGATCAAGAACTGCAGCTTCAACAT
CACCACCAGCGTGCGCGACAAGGTGCAGAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGG
TGCCCATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCAACACCAGCGTGATCACCC
AGGCCTGCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGCACC
GTGCAGTGCACCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTG
GCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCAACGACAACGCCAAGACCATCATCGT
GCAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAGAGCA
TCCACATCGGCCCGGCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGG
CCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTG
CGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGA
GATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTT
CAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCATCC
TGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCC
CCCCCATCCGCGGCCAGATCAAGTGACGAGCAATATTACCGGCCTGCTGCTGACCCGCGAC
GGCGGCACCAACAACAACCGCACCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACAT
GAAGGACAACCTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGGCGATCGAGCCCCTGGGCG
TGGCCCCCACCAGGCCAAGCGCCGCGTGCTGGTGACGCGGAGAAGCGCGCCGTGGGCCTGGGC
GCCCTGTTTCATCGGCTTCTGGGCGCCGCGGGAGCACCATGGGCGCCGCTCCGTGACCCTG
ACCGTGACGGCCCGCCAGCTGCTGAGCGGCATCGTGACGAGCAGAGAACCAACCTGCTGCGCGC
CATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCC
GCATCCTGGCCGTGGAGCGCTACCTGAAGGACAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGAC
CGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCC
TGATCTACAACCTGATCGAGATCGCCAGAACAGCAGGAGAAGAACGAGCAGGAGCTGCTG
GAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATC
CGCATCTTCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGC
ATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAG
CGCGGCCCGGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCA
GCAACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGT
TCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCC
GCCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACCTGCTGCAGTACTGGAGCCAGGAGCTG
AAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCG
CATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCA
GGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA

FIG. 52

(SEQ ID NO:65)

65/131

gp160.mod.US4.delV2

GAATTCCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGG
TGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGACCTGAACTGCACCGACAAGCTGACCGGCAGCACCAA
CGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCACCA
ACAGCACCAGCAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACTGCAGCTTCAAC
ATCGGCGCCCGGCCGCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCAA
GGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGA
AGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGACG
TGCACCACCGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGC
CGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGACCATCATCG
TGCAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAG
AGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACAT
CCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAGATCG
TGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAACAGCAGCAGC
GGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTG
CAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCA
AGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG
GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAA
TATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAACGACA
CCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTAC
AAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCGCCCCACCCAGGCCAAGCGCCG
CGTGGTGACGCGGAGAAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCCTGG
GCGCCGCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACAGGCCCGCCAG
CTGCTGAGCGGCATCGTGACGAGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCA
GCACCTGCTGACGCTGACCGTGTTGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCG
TGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTG
ATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGAT
CTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGA
TCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTG
GAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTA
CATCCGCATCTTCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTCCCG
TGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGC
CTGCCCCGCCAGCGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCG
CGACCGCGACCGCAGCAACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACC
TGCGCAGCCTGTGCCTGTTACGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGCC
CGCATCGTGGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACCT
GCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCG
CCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGC
GCCGTGATCCACATCCCCCGCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGA
TATCGGATCCTCTAGA

FIG. 53

(SEQ ID NO:66)

66 / 131

gp160.modUS4delV1/2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCCGGCCAGGCCTGCCC
CAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAA
GTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGCA
CCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAG
GAGATCGTGCTGCGCTCCGAGAACTTCAACGACAACGCCAAGACCATCATCGTGCAAGCTGAA
CGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAACACGCGTAAGAGCATCCACATCG
GCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCA
ACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAG
TTCGGCAACAACAAGACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTT
CCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCA GCTGTTCAACAGCAC
CTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCC
GCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATC
CGCGGCCAGATCAAGTGACGACGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCAC
CAACAACAACCGCACCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACA
ACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCCCCC
ACCCAGGCCAAGCGCCGCGTGGTGACGCGGAGAAGCGCGCCGTGGGCTGGGCGCCCTGTT
CATCGGCTTCCTGGGCGCCGCGGGAGCACCATGGGCGCCGCTCCGTGACCTGACCGTGCA
GGCCCCGCCAGCTGCTGAGCGGCATCGTGACGACGAGCAACAACCTGCTGCGCGCCATCGAGG
CCCAGCAGCACCTGCTGACGCTGACCGTGTTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTG
GCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCT
GATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCT
GGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTAC
AACCTGATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGG
ACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCT
TCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGA
ACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAGCGCGGC
CCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGCAACC
GCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGTTACGCT
ACCACCGCCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCGCCGCG
GCTGGGAGGCCCTGAAGTACTGGTGGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGC
AGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGCAACCGACCGCATCATC
GAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGGCCTG
GAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA

FIG. 54

(SEQ ID NO:67)

gp160.modUS4 del 128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCCCAAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
GGGGCAGGGAACTGCGAGACCAGCGTGATACCCAGGCCTGCCCCAAGGTGAGCTTCGAGCC
CATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTT
CAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCCG
TGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCC
GAGAACTTCACCGACAACGCCAAGACCATCATCGTGAGCTGAACGAGTCCGTGGAGATCAA
CTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTA
CGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACT
GGACCAACACCCTCGAGCAGATCGTGGAAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACC
ATCATCTTCAACAGCAGCAGCGGCGGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGC
GGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAG
GTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAA
CATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCA
GCAGCAATATTACCGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAAC
GACACCGAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTA
CAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCGCCCCACCCAGGCCAAGCGCCGCG
TGGTGACGCGGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCCG
CCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAGC
GGCATCGTGACGAGCAGAACAACTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCA
GCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTGA
AGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCACCGTG
CCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGAT
GGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCC
AGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGGCCAGCCTGTG
GAACTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGG
CCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTA
CAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAGCGCGGCCCGGACCGCCCCGAGGGCA
TCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGCAACCGCCTGGTGACGGCCTGCTG
GCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGTTAGCTACCACCGCCTGCGCGACCTG
CTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCGCGCGGCTGGGAGGCCCTGAAGTAC
TGGTGGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAA
CGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTT
CCGCGCCGTGATCCACATCCCCCGCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGA
TATCGGATCCTCTAGA

FIG. 55

(SEQ ID NO:68)

68 / 131

Env_US4_C4wt

GACACTATCATACTCCCATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGG
AAAAGCAATGTATGCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAG
GGCTGCTATTAACTAGAGATGGTGGT

FIG. 56

(SEQ ID NO:69)

Env_SF162_C4wt

GGAACATCACACTCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGG
AAAAGCAATGTATGCCCCTCCCATCAGAGGACAAATTAGATGCTCATCAAATATTACAG
GACTGCTATTAAACAAGAGATGGTGGT

FIG. 57

(SEQ ID NO:70)

Env_US4_C4mod

GACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGG
CAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAACATCACCG
GCCTGCTGCTGACCCGCGACGGCGGC

FIG. 58

(SEQ ID NO:71)

Env_SF162_C4mod

GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCAGGAGGTGGG
CAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCCGCTGCAGCAGCAACATCACCG
GCCTGCTGCTGACCCGCGACGGCGGC

FIG. 59

(SEQ ID NO:72)

69 / 131

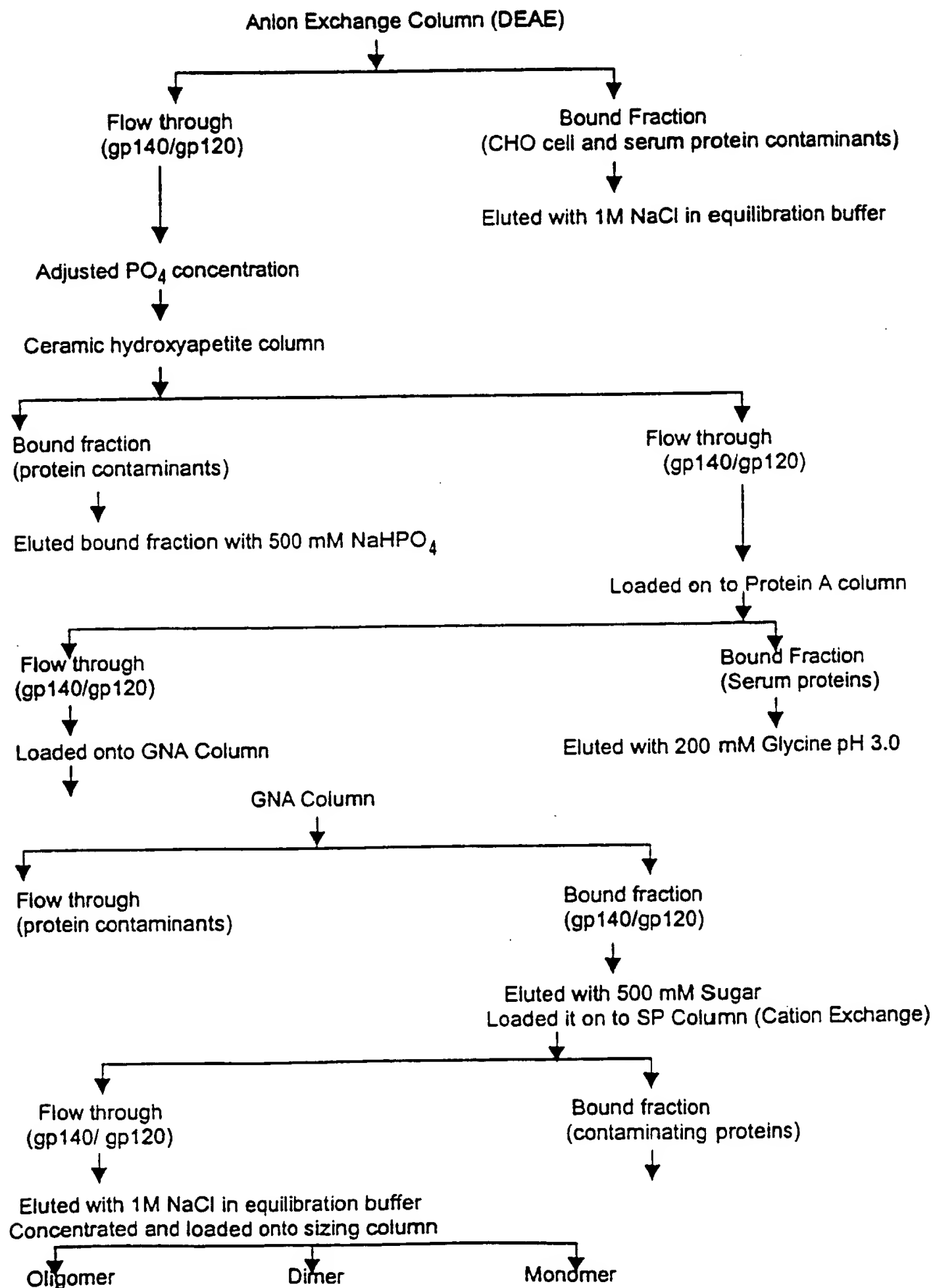


FIG. 60

70 / 131

gp160mod.us4.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTA CTACGGCGTG
CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAG
GCCGAGGCCCCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG
GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG
ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGGCACC
AACAGCACCAGCGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCACCACAGCACC
GACAGCTGGGAGAAGATGCCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACC
AGCGTGCGCGACAAGGTGCAGAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC
ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCAACACCAGCGTGATCACCAG
GCCTGCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC
GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGC
ACCGTGCAAGTGCAACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC
AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGACC
ATCATCGTGCAAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACAG
CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGC
GACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG
ATCGTGAGGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAACAGCAGC
AGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC
TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGAGGTGAACAAGACC
AAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG
GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAAGCAAT
ATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC
GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTACAAG
TACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCGCCCCACCCAGGCCAAGCGCCGCGTG
GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGCAAGGCCCGCCAGCTGCTG
AGCGGCATCGTGCAAGCAGCAGACAACCTGCTGCGCGCCATCGAGGCCCGCAGCAGCCTG
CTGCAGCTGACCGTGTTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGC
TACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACC
ACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAAC
ATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTG
ATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAG
TGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACCTGGCTGTGGTACATCCGCATCTTC
ATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTG
AACC GCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCCAGCGC
GGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGC
AACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTG
TTCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGCGCCCGCATCGTGAGGCTGCTG
GGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACTGCTGCAGTACTGGAGCCAG
GAGCTGAAGAGCAGCGCGCTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGC
ACCGACCGCATCATCGAGATCGTGAGCGCATCTTCCGCGCCGTGATCCACATCCCCCGC
CGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGAGAATTC

FIG. 61A

(SEQ ID NO:73)

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CGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGC
TTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTT
GGCAATGTGAGGGCCCCGGAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTT
TCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTG
GAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCA
CCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCG
GCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCC
TCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATGGGATCT
GATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTA
GGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATAATACCATGGGCGC
CCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGGGAGAAGATCCGCTGCGCCC
CGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGGCCAGCCGCGAGCTGGAGCG
CTTCGCGGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGCTGCCGCCAGATCCTGGGCCA
GCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGCAGCCTGTACAACACCGTGGC
CACCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGACACCAAGGAGGCCCTGGAGAA
GATCGAGGAGGAGCAGAACAAAGTCCAAGAAGAAGGCCAGCAGGCCGCCGCCGCCGCCG
CACCGGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCAGGGCCA
GATGGTGCACCAGGCCATCAGCCCCCGCACCTGAACGCCTGGGTGAAGGTGGTGGAGGA
GAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGAGCGAGGGCGCCACCCC
CCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCT
GAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCGCGTGCACCCCGTGCACGCCGG
CCCCATCGCCCCCGGCCAGATGCGCGAGCCCCCGCGGCAGCGACATCGCCGGCACCAACAG
CACCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGGCGAGAT
CTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTGCAGGATGTACAGCCCCACCA
CATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCGCGACTACGTGGACCGCTTCTA
CAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGACCGAGACCCT
GCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCCGCGGC
CACCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCGGCCACAAGGCCCG
CGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCAGCGCGG
CAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACAC
CGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCCGCGAGGGCCA
CCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCCAGCTA
CAAGGGCCGCCCGGCAACTTCTGCGAGAGCCGCCCGAGCCACCGCCCCCGAGGA
GAGCTTCCGCTTCGGCGAGGAGAAGACCCCCAGCCAGAAGCAGGAGCCCATCGACAA
GGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTTCGGCAACGACCCAGCAGCCAGTA
AGAATTGAGACTCGAGCAAGTCTAGA

FIG. 61B

(SEQ ID NO:73)

72 / 131

gp160mod.SF162.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCGTGGAGAAGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCCTAC
GACACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGATCGTGCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGACCCCTGCACTGCACCAACCTGAAGAACGCCACCAACAC
CAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAGATCAAGAACTGCAGCTTCAAGG
TGACCACCAGCATCCGCAACAAGATGCAGAAGGAGTACGCCCTGTTCTACAAGCTGGAC
GTGGTGCCCATCGACAACGACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGT
GATCACCAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCC
CCGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCGGCCCTGC
ACCAACGTGAGCACCGTGACGTGCACCCACGGCATCCGCCCGTGGTGAGCACCCAGCT
GCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGTGGTGATCCGCAGCGAGAACTTCACCG
ACAACGCCAAGACCATCATCGTGACGTGAAGGAGAGCGTGAGATCAACTGCACCCGC
CCCAACAACAACACCCGCAAGAGCATCACCATCGGCCCGCGCGCCTTCTACGCCAC
CGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGGCGAGAAGTGGA
ACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTTCGGCAACAAGACCATC
GTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGTGATGCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACAGCACCCAGCTGTTCAACAGCACCTGGAACAACACCA
TCGGCCCCAACAACACCAACGGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATC
AACCGCTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCCGCCAGATCCG
CTGCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAGATCAGCA
ACACCACCGAGATCTTCCGCCCGGCGGCGGCGACATGCGCGACAACCTGGCGCAGCGAG
CTGTACAAGTACAAGGTGGTGAAGATCGAGCCCCTGGGCGTGGCCCCACCAAGGCCAA
GCGCCGCGTGGTGACGCGGAGAAGCGCGCGCGTGACCCCTGGGCGCCATGTTCCCTGGGCT
TCCTGGGCGCCGCGGCGAGCACCATGGGCGCCCCGAGCCTGACCCCTGACCGTGACGGC
CGCCAGCTGCTGAGCGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGC
CCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCGTGC
TGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGC
AAGCTGATCTGCACCACCGCCGTGCCCTGGAACGCCAGCTGGAGCAACAAGAGCCTGGA
CCAGATCTGGAACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCA
ACCTGATCTACACCCTGATCGAGGAGAGCCAGAACCAGCAGGAGAAGAACGAGCAGGAG
CTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAAGTGGCT
GTGGTACATCAAGATCTTCATCATGATCGTGCGCGGCGCTGGTGGGCCTGCGCATCGTGT
TCACCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCCTGAGCTTCCAG
ACCCGCTTCCCCGCCCCCGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGG
CGAGCGCGACCGCGACCGCAGCAGCCCCCTGGTGACGGCCTGCTGGCCCTGATCTGGG
ACGACCTGCGCAGCCTGTGCCTGTTTCACTACCGCCCTGCGCGACCTGATCCTGATC
GCCGCCCGCATCGTGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGGG
CAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGCGCCGTGAGCCTGTTCGACG
CCATCGCCATCGCGTGGCCGAGGGCACCGACCGCATCATCGAGGTGGCCAGCGCATC
GGCCGCGCCTTCTGACATCCCCCGCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCT

FIG. 62A

(SEQ ID NO:74)

GTAAC TCGAGCAAGTCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCCTCCCC
CCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATAT
GTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTG
TCTTCTTGACGAGCATTCCTAGGGGTCTTTCCTCTCGCCAAAGGAATGCAAGGTCTG
TTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGT
AGCGACCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAA
AGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGT
TGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAA
GGATGCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCT
TTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTG
GTTTTCTTTTGAAAAACACGATAATACCATGGGCGCCCGCGCCAGCGTGCTGAGCGGCG
GCGAGCTGGACAAAGTGGGAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAG
CTGAAGCACATCGTGTGGGCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCT
GCTGGAGACCAGCGAGGGCTGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGA
CCGGCAGCGAGGAGCTGCGCAGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCAC
CAGCGCATCGACGTCAAGGACACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAA
CAAGTCCAAGAAGAAGGCCCAGCAGGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCC
AGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCC
ATCAGCCCCCGCACCCCTGAACGCCTGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCC
CGAGGTGATCCCCATGTTTCAGCGCCCTGAGCGAGGGCGCCACCCCCCAGGACCTGAACA
CGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGAGACCATC
AACGAGGAGGCCGCCGAGTGGGACCGCGTGCACCCCGTGCACGCCGGCCCCCATCGCCCC
CGGCCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGGCACCAACAGCACCTTGCAGG
AGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGGCGAGATCTACAAGCGG
TGGATCATCCTGGGCCTGAACAAGATCGTGCGGATGTACAGCCCCACCAGCATCCTGGA
CATCCGCCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGCTTCTACAAGACCC
TGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGAACCGAGACCCTGCTGGTG
CAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCCGCGGGCCACCCT
GGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCGGCCACAAGGCCCGCGTGC
TGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCAGCGCGGCAAC
TTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACACCGC
CAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCCGCGAGGGGCCACC
AGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCCAGCTAC
AAGGGCCGCCCGGCCAACTTCTGCAGAGCCGCCCGAGCCCACCGCCCCCCCCCGAGGA
GAGCTTCCGCTTCGGCGAGGAGAAGACCACCCCGCCAGACAGCAGGAGCCCATCGACA
AGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTCCGGCAACGACCCCGAGCAGCCAG
TAAGAAATTCAGACTCGAGCAAGTCTAGA

FIG. 62B
(SEQ ID NO:74)

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gp160modUS4.delV1/V2.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTCGCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG
CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAG
GCCGAGGCCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG
GAGGTGAACCTGACCAACGTGACCGAGAATTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCC
GGCCAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCC
GGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAAC
GTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTG
AACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAATTACCGACAACGCC
AAGACCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAC
AACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATC
ATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTC
GAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTC
TTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAAC
AAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATG
TGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGC
AGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCCACCAACAACAACCGCACCAAC
GACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTG
TACAAGTACAAGGTGGTGGCATCGAGCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGC
CGCGTGGTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCCTG
GGCGCCGCGGGAGCACCATGGGCGCCGCTCCGTGACCTGACCGTGACGGCCCGCCAG
CTGCTGAGCGGCATCGTGCAAGCAGCAGAACCAACCTGCTGCGCGCCATCGAGGCCCGAGCAG
CACCTGCTGCAAGTGAACGAGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTG
GAGCGCTACCTGAAGGACAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATC
TGCACCACCAACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGG
GACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTAC
AACCTGATCGAGATCGCCCCAGAACCAGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTG
GACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATCCGC
ATCTTCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGC
ATCGTGAAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCGCTGCCCGCC
CAGCGCGGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC
CGCAGCAACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTG
TGCCTGTTTCAGCTACCAACCGCCTGCGCGACCTGCTGCTGATCGTGGCCCCGCATCGTGGAG
CTGCTGGGCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACCTGCTGCAGTACTGG
AGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC
GAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATC
CCCCGCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA
GAATTCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGA
AGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCG
TCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGG
GGTCTTTCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTT

FIG. 63A

(SEQ ID NO:75)

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CCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAAC
CCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCA
AAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGG
CTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATG
GGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAA
CGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATAATAACCAT
GGGCGCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGGGAGAAGATCCGCCT
GCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGGCCAGCCGCGAGCT
GGAGCGCTTCGCCGTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCCGCCAGATCCT
GGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGCAGCCTGTACAACAC
CGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGACACCAAGGAGGCCCT
GGAGAAGATCGAGGAGGAGCAGAAACAAGTCCAAGAAGAAGGCCAGCAGGCCGCGCGCCG
CGCCGGCACCGGCAACAGCAGCCAGGTGAGCCAGAATAACCCCATCGTGCAGAACCTGCA
GGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCTGAACGCCTGGGTGAAGGTGGT
GGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGAGCGAGGGCGC
CACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCA
GATGCTGAAGGAGACCATCAACGAGGAGGCCGCGCGAGTGGGACCGCGTGCACCCCGTGCA
CGCCGGCCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGGCAC
CACCAGCACCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGG
CGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTGCAGGATGTACAGCCC
CACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCG
CTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGACCGA
GACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCC
CGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCCGGCCACAA
GGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCA
GCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGG
CCACACCGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGCA
GGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCC
CAGCTACAAGGGCCGCCCCGGCAACTTCTGCGAGCCGCCCCGAGCCACCGCCCCCCC
CGAGGAGAGCTTCCGCTTCGGCGAGGAGAAGACCACCCCGAGCCAGAAGCAGGAGCCCAT
CGACAAGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTCCGGCAACGACCCCGAGCAG
CCAGTAAGAATTTCAGACTCGAGCAAGTCTAGA

FIG. 63B

(SEQ ID NO:75)

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gp160.modSF162.delV2.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTCGCCCAGCGCCGTGGAGAAGCTGTGGGTGACCGTGTACTACGGCGTG
CCCGTGTGGAAGGAGGCCACCAACCCCTGTTCTGCGCCAGCGACGCCAAGGCCTACGAC
ACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCCCCAG
GAGATCGTGTGGAAGACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG
ACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGAACGCCACCAACACCAAGAGC
AGCAACTGGAAGGAGATGGACCGCGGCGAGATCAAGAACTGCAGCTTCAAGGTGGGCGCC
GGCAAGCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCCAAGGTGAGCTTC
GAGCCCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAACGAC
AAGAAGTTCAACGGCAGCGGCCCTGCACCAACGTGAGCACCGTGAGTGCACCCACGGC
ATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGTG
GTGATCCGCAGCGAGAATTACCCGACAACGCCAAGACCATCATCGTGAGCTGAAGGAG
AGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACCCGCAAGAGCATCACCATCGGC
CCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTGC
AACATCAGCGGCGAGAAGTGGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCC
CAGTTCGGCAACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGTG
ATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCCAGCTGTTCAAC
AGCACCTGGAACAACACCATCGGCCCAACAACACCAACGGCACCATCACCTGCCCTGC
CGCATCAAGCAGATCATCAACCGCTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCC
ATCCGCGGCCAGATCCGCTGCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGC
GGCAAGGAGATCAGCAACACCACCGAGATCTTCCGCCCCGGCGGCGGCGACATGCGCGAC
AACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCCTGGGCGTGGCC
CCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGCGCGCCGTGACCCCTGGGCGCC
ATGTTCTTGGGCTTCCTGGGCGCCGCGGCGAGCACCATGGGCGCCCGCAGCCTGACCCTG
ACCGTGCAGGCCCCGCGAGCTGCTGAGCGGCATCGTGAGCAGCAGACAACCTGCTGCGC
GCCATCGAGGCCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAG
GCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGC
TGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGGAACGCCAGCTGGAGCAACAAG
AGCCTGGACCAGATCTGGAACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGACAAC
TACACCAACCTGATCTACACCCTGATCGAGGAGAGCCAGAACCAGCAGGAGAAGAACGAG
CAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAAG
TGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGGCGGCCTGGTGGGCCTGCGCATC
GTGTTACCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCCTGAGCTTC
CAGACCCGCTTCCCCGCCCCCGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGC
GGCGAGCGCGACCGCGACCGCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGG
GACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCACCGCCTGCGCGACCTGATCCTGATC
GCCGCCCGCATCGTGGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGGGC
AACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGCGCCGTGAGCCTGTTTCGACGCC
ATCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGGTGGCCCAGCGCATCGGC
CGCGCCTTCTTGCACATCCCCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAA
CTCGAGCAAGTCTAGAGAAATCCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCCC
TAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATT
TTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTT

FIG. 64A

(SEQ ID NO:76)

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GACGAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGT
CGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCT
TTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGT
ATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGT
GGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAA
GGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTA
GTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAA
AACACGATAATACCATGGGCGCCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGT
GGGAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGT
GGGCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGG
GCTGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGC
GCAGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGG
ACACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAAGTCCAAGAAGAAGGCC
AGCAGGCCCGCCGCGCCGCGCCGGCACC GGCAACAGCAGCCAGGTGAGCCAGAACTACCCCA
TCGTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCTGAACG
CCTGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCATGTTCAGCG
CCCTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCC
ACCAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACC
GCGTGCACCCCGTGCACGCCGGCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCA
GCGACATCGCCGGCACCAACAGCACCTTGCAGGAGCAGATCGGCTGGATGACCAACAACC
CCCCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCG
TGCGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCC
GCGACTACGTGGACCGCTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGA
AGAAGTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCC
TGAAGGCTCTCGGCCCCCGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGG
GCGGCCCCCGGCCACAAGGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGG
CGACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCA
ACTGCGGCAAGGAGGGCCACACCGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCT
GGCGCTGCGGCCCGGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCC
TGGGCAAGATCTGGCCCAGCTACAAGGGCCGCCCCGGCAACTTCTTGCAGAGCCGCCCCG
AGCCCACCGCCCCCCCCGAGGAGAGCTTCCGCTTCGGCGAGGAGAAGACCACCCCAGCC
AGAAGCAGGAGCCCATCGACAAGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTCG
GCAACGACCCCAGCAGCCAGTAAGAATTCACTCGAGCAAGTCTAGA

FIG. 64B

(SEQ ID NO:76)

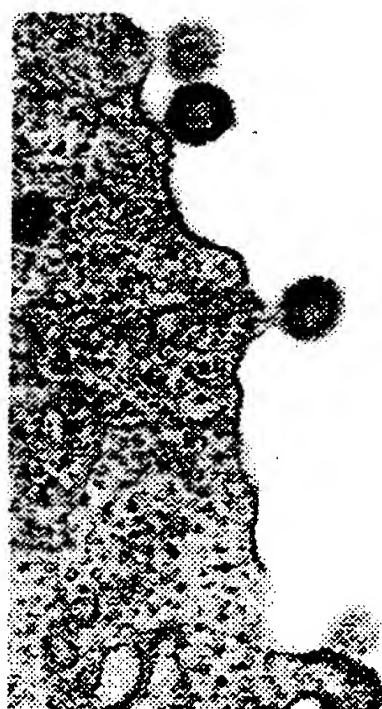


FIG. 65C



FIG. 65B



FIG. 65A

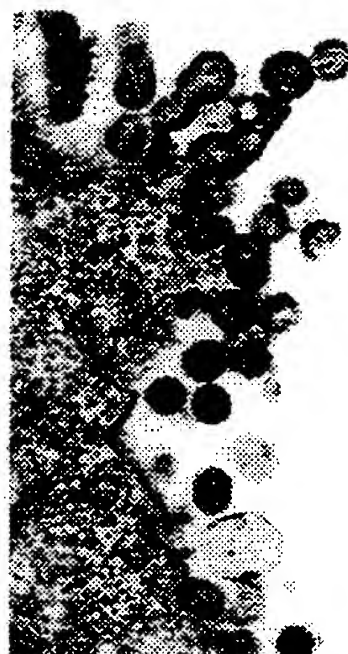


FIG. 65F



FIG. 65E



FIG. 65D

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	1		50
gp160.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp160.modSF162.delV2	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp160.modSF162.delV1V2	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.mut.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.mut7.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.mut8.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp120.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
Consensus	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	51
gp160.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
gp160.modSF162.delV2	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
gp160.modSF162.delV1V2	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.mut.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.mut7.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.mut8.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
gp120.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	
Consensus	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCCCAGCGCCGTGGAGAAGCTGTGGG	101
gp160.modSF162	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
gp160.modSF162.delV2	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
gp160.modSF162.delV1V2	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
gp140.modSF162	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
gp140.mut.modSF162	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
gp140.mut7.modSF162	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
gp140.mut8.modSF162	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
gp120.modSF162	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	
Consensus	(101)	TGACCGTGTAACGGCGTGCCCGTGTGGAAGAGGCCACCAACCCCTG	150

FIG. 66A-1

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gp120.modSF162	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACACATGGTGGAG	350
Consensus	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACACATGGTGGAG	
gp160.modSF162	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp160.modSF162.delV2	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp160.modSF162.delV1V2	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp140.modSF162	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp140.mut.modSF162	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp140.mut7.modSF162	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp140.mut8.modSF162	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp120.modSF162	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
Consensus	(301)	CAGATGCACGAGGACATCATCAGCCTGTGGACCAGAGCCTGAAGCCCCTG	
gp160.modSF162	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	400
gp160.modSF162.delV2	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
gp160.modSF162.delV1V2	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
gp140.modSF162	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
gp140.mut.modSF162	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
gp140.mut7.modSF162	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
gp140.mut8.modSF162	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
gp120.modSF162	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
Consensus	(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA	
gp160.modSF162	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	450
gp160.modSF162.delV2	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	
gp160.modSF162.delV1V2	(375)	-----	
gp140.modSF162	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	
gp140.mut.modSF162	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	
gp140.mut7.modSF162	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	
gp140.mut8.modSF162	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	
gp120.modSF162	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	
Consensus	(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGGAG	

FIG. 66A-3

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gp160.modSF162	(451)	ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA	500
gp160.modSF162.delV2	(451)	ATCAAGAACTGCAGCTTCAAGGTGGC-----	
gp160.modSF162.delV1V2	(376)	-----GGC-----	
gp140.modSF162	(451)	ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA	
gp140.mut.modSF162	(451)	ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA	
gp140.mut7.modSF162	(451)	ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA	
gp140.mut8.modSF162	(451)	ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA	
gp120.modSF162	(451)	ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA	
Consensus	(451)	ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA	500
gp160.modSF162	(501)	GAAGGAGTACGCCCTGTCTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	550
gp160.modSF162.delV2	(478)	-----GCC-----GG-----	
gp160.modSF162.delV1V2	(379)	-----GCC-----GG-----	
gp140.modSF162	(501)	GAAGGAGTACGCCCTGTCTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	
gp140.mut.modSF162	(501)	GAAGGAGTACGCCCTGTCTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	
gp140.mut7.modSF162	(501)	GAAGGAGTACGCCCTGTCTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	
gp140.mut8.modSF162	(501)	GAAGGAGTACGCCCTGTCTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	
gp120.modSF162	(501)	GAAGGAGTACGCCCTGTCTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	
Consensus	(501)	GAAGGAGTACGCCCTGTCTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	550
gp160.modSF162	(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAACAGCGTGATCACCCAG	600
gp160.modSF162.delV2	(492)	-----CAAGCTGATCAACTGCAACACCAACAGCGTGATCACCCAG	
gp160.modSF162.delV1V2	(384)	-----CAACTGCCAGACCAACAGCGTGATCACCCAG	
gp140.modSF162	(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAACAGCGTGATCACCCAG	
gp140.mut.modSF162	(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAACAGCGTGATCACCCAG	

FIG. 66A-4

FIG. 66A-5

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gp160.modSF162	(751)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	800
gp160.modSF162.delV2	(670)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	
gp160.modSF162.delV1V2	(562)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	
gp140.modSF162	(751)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	
gp140.mut.modSF162	(751)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	
gp140.mut7.modSF162	(751)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	
gp140.mut8.modSF162	(751)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	
gp120.modSF162	(751)	GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	
Consensus		GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT	850
gp160.modSF162	(801)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
gp160.modSF162.delV2	(720)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
gp160.modSF162.delV1V2	(612)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
gp140.modSF162	(801)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
gp140.mut.modSF162	(801)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
gp140.mut7.modSF162	(801)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
gp140.mut8.modSF162	(801)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
gp120.modSF162	(801)	GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	
Consensus		GGTGATCCGCAGCGAGAACTTACCCGACAAACGCCAAGACCATCATCGTGC	900
gp160.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCCAACAACACACC	
gp160.modSF162.delV2	(770)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCCAACAACACACC	
gp160.modSF162.delV1V2	(662)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCCAACAACACACC	

FIG. 66A-6

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gp140.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCAACCGCGCCCAACAACAACACC	901
gp140.mut.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCAACCGCGCCCAACAACAACACC	
gp140.mut7.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCAACCGCGCCCAACAACAACACC	
gp140.mut8.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCAACCGCGCCCAACAACAACACC	
gp120.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCAACCGCGCCCAACAACAACACC	
Consensus		AGCTGAAGGAGAGCGTGGAGATCAACTGCAACCGCGCCCAACAACAACACC	950
gp160.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
gp160.modSF162.delV2	(820)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
gp160.modSF162.delV1V2	(712)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
gp140.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
gp140.mut.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
gp140.mut7.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
gp140.mut8.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
gp120.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	
Consensus		CGCAAGAGCATCACCATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGGA	951
gp160.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	1000
gp160.modSF162.delV2	(870)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	
gp160.modSF162.delV1V2	(762)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	
gp140.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	
gp140.mut.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	
gp140.mut7.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	
gp140.mut8.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	
gp120.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	
Consensus		CATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGCGGAGAAAGT	

FIG. 66A-7

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gp160.modSF162	(1001)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	1050
gp160.modSF162.delV2	(920)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	
gp160.modSF162.delV1V2	(812)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	
gp140.modSF162	(1001)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	
gp140.mut.modSF162	(1001)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	
gp140.mut7.modSF162	(1001)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	
gp140.mut8.modSF162	(1001)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	
gp120.modSF162	(1001)	GGAACAACACCCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCGGC	
Consensus			1100
gp160.modSF162	(1051)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
gp160.modSF162.delV2	(970)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
gp160.modSF162.delV1V2	(862)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
gp140.modSF162	(1051)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
gp140.mut.modSF162	(1051)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
gp140.mut7.modSF162	(1051)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
gp140.mut8.modSF162	(1051)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
gp120.modSF162	(1051)	AACAAGACCATCGTGTTC AAGCAGAGCAGCGGCGGACCCCGAGATCGT	
Consensus			1150
gp160.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp160.modSF162.delV2	(1020)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp160.modSF162.delV1V2	(912)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.mut.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.mut7.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.mut8.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp120.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
Consensus			1200
gp160.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCACCATCGGGCCCCAACACCAAC	

FIG. 66A-8

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gp160.modSF162.delV2	(1070)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	1250
gp160.modSF162.delV1V2	(962)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	
gp140.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	
gp140.mut.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	
gp140.mut7.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	
gp140.mut8.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	
gp120.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	
Consensus	(1151)	AGCTGTTCAACAGCACCTGGAAACAACACCATCGGGCCCCAACAAACACCAAC	1250
gp160.modSF162	(1201)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	1251
gp160.modSF162.delV2	(1120)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	
gp160.modSF162.delV1V2	(1012)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	
gp140.modSF162	(1201)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	
gp140.mut.modSF162	(1201)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	
gp140.mut7.modSF162	(1201)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	
gp140.mut8.modSF162	(1201)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	
gp120.modSF162	(1201)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	
Consensus	(1201)	GGACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA	1251
gp160.modSF162	(1251)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	1300
gp160.modSF162.delV2	(1170)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	
gp160.modSF162.delV1V2	(1062)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	
gp140.modSF162	(1251)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	
gp140.mut.modSF162	(1251)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	
gp140.mut7.modSF162	(1251)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	
gp140.mut8.modSF162	(1251)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	
gp120.modSF162	(1251)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	
Consensus	(1251)	GGAGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGGGGCCAGATCCGCT	

FIG. 66A-9

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gp160.modSF162	(1301)	GCAGCAGCAACATCACC	1350
gp160.modSF162.delV2	(1220)	GCAGCAGCAACATCACC	
gp160.modSF162.delV1V2	(1112)	GCAGCAGCAACATCACC	
gp140.modSF162	(1301)	GCAGCAGCAACATCACC	
gp140.mut.modSF162	(1301)	GCAGCAGCAACATCACC	
gp140.mut7.modSF162	(1301)	GCAGCAGCAACATCACC	
gp140.mut8.modSF162	(1301)	GCAGCAGCAACATCACC	
gp120.modSF162	(1301)	GCAGCAGCAACATCACC	
Consensus	(1301)	GCAGCAGCAACATCACC	
gp160.modSF162	(1351)	ATCAGCAACACCA	1400
gp160.modSF162.delV2	(1270)	ATCAGCAACACCA	
gp160.modSF162.delV1V2	(1162)	ATCAGCAACACCA	
gp140.modSF162	(1351)	ATCAGCAACACCA	
gp140.mut.modSF162	(1351)	ATCAGCAACACCA	
gp140.mut7.modSF162	(1351)	ATCAGCAACACCA	
gp140.mut8.modSF162	(1351)	ATCAGCAACACCA	
gp120.modSF162	(1351)	ATCAGCAACACCA	
Consensus	(1351)	ATCAGCAACACCA	
gp160.modSF162	(1401)	CAACTGGCGCAGCGAG	1450
gp160.modSF162.delV2	(1320)	CAACTGGCGCAGCGAG	
gp160.modSF162.delV1V2	(1212)	CAACTGGCGCAGCGAG	
gp140.modSF162	(1401)	CAACTGGCGCAGCGAG	
gp140.mut.modSF162	(1401)	CAACTGGCGCAGCGAG	
gp140.mut7.modSF162	(1401)	CAACTGGCGCAGCGAG	
gp140.mut8.modSF162	(1401)	CAACTGGCGCAGCGAG	
gp120.modSF162	(1401)	CAACTGGCGCAGCGAG	
Consensus	(1401)	CAACTGGCGCAGCGAG	

FIG. 66A-10

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gp160.modSF162	(1451)	TGGGCGTG	1451	1500
gp160.modSF162.delV2	(1370)	TGGGCGTG		
gp160.modSF162.delV1V2	(1262)	TGGGCGTG		
gp140.modSF162	(1451)	TGGGCGTG		
gp140.mut.modSF162	(1451)	TGGGCGTG		
gp140.mut7.modSF162	(1451)	TGGGCGTG		
gp140.mut8.modSF162	(1451)	TGGGCGTG		
gp120.modSF162	(1451)	TGGGCGTG		
Consensus	(1451)	TGGGCGTG		
gp160.modSF162	(1501)	CGGCGCGT	1501	1550
gp160.modSF162.delV2	(1420)	CGGCGCGT		
gp160.modSF162.delV1V2	(1312)	CGGCGCGT		
gp140.modSF162	(1501)	CGGCGCGT		
gp140.mut.modSF162	(1501)	AGCGCCGT		
gp140.mut7.modSF162	(1501)	AGCGCCGT		
gp140.mut8.modSF162	(1501)	AGCGCCGT		
gp120.modSF162	(1501)	CGC----		
Consensus	(1501)	CGCGCCGT		
gp160.modSF162	(1551)	CAGCACCAT	1551	1600
gp160.modSF162.delV2	(1470)	CAGCACCAT		
gp160.modSF162.delV1V2	(1362)	CAGCACCAT		
gp140.modSF162	(1551)	CAGCACCAT		
gp140.mut.modSF162	(1551)	CAGCACCAT		
gp140.mut7.modSF162	(1551)	CAGCACCAT		
gp140.mut8.modSF162	(1551)	CAGCACCAT		
gp120.modSF162	(1513)	-----		
Consensus	(1551)	CAGCACCAT		

FIG. 66A-11

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gp160.modSF162	(1601)	1601	TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	1650
gp160.modSF162.delV2	(1520)		TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	
gp160.modSF162.delV1V2	(1412)		TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	
gp140.modSF162	(1601)		TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	
gp140.mut.modSF162	(1601)		TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	
gp140.mut7.modSF162	(1601)		TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	
gp140.mut8.modSF162	(1601)		TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	
gp120.modSF162	(1513)		-----	
Consensus	(1601)		TGCTGAGCGGCATCGTGCAGCAGCAGAAACCTTGCTGCGGCCATCGAG	1700
gp160.modSF162	(1651)	1651	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	
gp160.modSF162.delV2	(1570)		GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	
gp160.modSF162.delV1V2	(1462)		GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	
gp140.modSF162	(1651)		GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	
gp140.mut.modSF162	(1651)		GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	
gp140.mut7.modSF162	(1651)		GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	
gp140.mut8.modSF162	(1651)		GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	
gp120.modSF162	(1513)		-----	
Consensus	(1651)		GCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGCATCAAGCAGCTGCA	1750
gp160.modSF162	(1701)	1701	GGCCCGCGTGTGCGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG	
gp160.modSF162.delV2	(1620)		GGCCCGCGTGTGCGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG	
gp160.modSF162.delV1V2	(1512)		GGCCCGCGTGTGCGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG	
gp140.modSF162	(1701)		GGCCCGCGTGTGCGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG	
gp140.mut.modSF162	(1701)		GGCCCGCGTGTGCGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG	
gp140.mut7.modSF162	(1701)		GGCCCGCGTGTGCGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG	
gp140.mut8.modSF162	(1701)		GGCCCGCGTGTGCGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG	

FIG. 66A-12

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-----
gp120.modSF162 (1513)
Consensus (1701) GGGCCGGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG
1751 1800
gp160.modSF162 (1751) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp160.modSF162.delV2 (1670) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp160.modSF162.delV1V2 (1562) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp140.modSF162 (1751) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp140.modSF162 (1751) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp140.modSF162 (1751) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp140.modSF162 (1751) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp140.modSF162 (1751) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
gp120.modSF162 (1513) -----
Consensus (1751) GCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGG
1801 1850
gp160.modSF162 (1801) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
gp160.modSF162.delV2 (1720) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
gp160.modSF162.delV1V2 (1612) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
gp140.modSF162 (1801) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
gp140.modSF162 (1801) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
gp140.modSF162 (1801) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
gp140.modSF162 (1801) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
gp120.modSF162 (1513) -----
Consensus (1801) AACGCCAGCTGGAGCAACAAGAGCCTGGACCAGATCTGGAACAACATGAC
1851 1900
gp160.modSF162 (1851) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
gp160.modSF162.delV2 (1770) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
gp160.modSF162.delV1V2 (1662) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
gp140.modSF162 (1851) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
gp140.modSF162 (1851) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
gp140.modSF162 (1851) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
gp140.modSF162 (1851) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
gp120.modSF162 (1513) -----
Consensus (1851) CTGGATGGAGTGGGAGCGCGAGATCGACAACCTAACCTGATCTACA
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FIG. 66A-13

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		1950
	1901	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp160.modSF162	(1901)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp160.modSF162.delV2	(1820)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp160.modSF162.delV1V2	(1712)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp140.modSF162	(1901)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp140.mut.modSF162	(1901)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp140.mut7.modSF162	(1901)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp140.mut8.modSF162	(1901)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
gp120.modSF162	(1513)	-----
Consensus	(1901)	CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGAGAAACGAGCAGGAGCTG
	1951	2000
gp160.modSF162	(1951)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
gp160.modSF162.delV2	(1870)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
gp160.modSF162.delV1V2	(1762)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
gp140.modSF162	(1951)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
gp140.mut.modSF162	(1951)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
gp140.mut7.modSF162	(1951)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
gp140.mut8.modSF162	(1951)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
gp120.modSF162	(1513)	-----
Consensus	(1951)	CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACTGGTTCGACATCAGCAA
	2001	2050
gp160.modSF162	(2001)	GTGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGCGGCCCTGGTGG
gp160.modSF162.delV2	(1920)	GTGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGCGGCCCTGGTGG
gp160.modSF162.delV1V2	(1812)	GTGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGCGGCCCTGGTGG
gp140.modSF162	(2001)	GTGGCTGTGGTACATCTAACTCGAG-----
gp140.mut.modSF162	(2001)	GTGGCTGTGGTACATCTAACTCGAG-----

FIG. 66A-14

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gp140.mut7.modSF162	(2001)	GTGGCTGTGGTACATCTAACTCGAG-----	2100
gp140.mut8.modSF162	(2001)	GTGGCTGTGGTACATCTAACTCGAG-----	
gp120.modSF162	(1513)	-----	
Consensus	(2001)	GTGGCTGTGGTACATCTAACTCGAG	
gp160.modSF162	2051		
gp160.modSF162.delV2	(2051)	GCCTGGCATCGTGTTCACCCGTGCTGAGCATCGTGAACCGCGTGCGCCAG	
gp160.modSF162.delV1V2	(1970)	GCCTGGCATCGTGTTCACCCGTGCTGAGCATCGTGAACCGCGTGCGCCAG	
gp140.modSF162	(1862)	GCCTGGCATCGTGTTCACCCGTGCTGAGCATCGTGAACCGCGTGCGCCAG	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2051)	-----	
gp160.modSF162	2101		
gp160.modSF162.delV2	(2101)	GGCTACAGCCCCCTGAGCTTCCAGACCCCGCTTCCCCCGCCCCCGCGGCC	2150
gp160.modSF162.delV1V2	(2020)	GGCTACAGCCCCCTGAGCTTCCAGACCCCGCTTCCCCCGCCCCCGCGGCC	
gp140.modSF162	(1912)	GGCTACAGCCCCCTGAGCTTCCAGACCCCGCTTCCCCCGCCCCCGCGGCC	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2101)	-----	
gp160.modSF162	2151		
gp160.modSF162.delV2	(2151)	CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGGAGCGCGGACCGCGACC	2200
gp160.modSF162.delV1V2	(2070)	CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGGAGCGCGGACCGCGACC	
gp140.modSF162	(1962)	CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGGAGCGCGGACCGCGACC	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2151)	-----	

FIG. 66A-15

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gp160.modSF162	(2201)	GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG	2250
gp160.modSF162.delV2	(2120)	GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG	
gp160.modSF162.delV1V2	(2012)	GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2201)	-----	
gp160.modSF162	(2251)	CGCAGCCTGTGCCCTGTTTCAGCTACCACCGCCTGCGCGACCTGATCCTGAT	2300
gp160.modSF162.delV2	(2170)	CGCAGCCTGTGCCCTGTTTCAGCTACCACCGCCTGCGCGACCTGATCCTGAT	
gp160.modSF162.delV1V2	(2062)	CGCAGCCTGTGCCCTGTTTCAGCTACCACCGCCTGCGCGACCTGATCCTGAT	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2251)	-----	
gp160.modSF162	(2301)	CGCCGCCCGCATCGTGGAGCTGCTGGGCCCGCCGCGGCTGGGAGGCCCTGA	2350
gp160.modSF162.delV2	(2220)	CGCCGCCCGCATCGTGGAGCTGCTGGGCCCGCCGCGGCTGGGAGGCCCTGA	
gp160.modSF162.delV1V2	(2112)	CGCCGCCCGCATCGTGGAGCTGCTGGGCCCGCCGCGGCTGGGAGGCCCTGA	

FIG. 66A-16

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gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2301)	-----	
		2351	2400
gp160.modSF162	(2351)	AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC	
gp160.modSF162.delV2	(2270)	AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC	
gp160.modSF162.delV1V2	(2162)	AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2351)	-----	
		2401	2450
gp160.modSF162	(2401)	GCCGTGAGCCTGTTTCGACGCCCATCGCCATCGCCGTGGCCGAGGGCACCCGA	
gp160.modSF162.delV2	(2320)	GCCGTGAGCCTGTTTCGACGCCCATCGCCATCGCCGTGGCCGAGGGCACCCGA	
gp160.modSF162.delV1V2	(2212)	GCCGTGAGCCTGTTTCGACGCCCATCGCCATCGCCGTGGCCGAGGGCACCCGA	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2401)	-----	

FIG. 66A-17

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gp160.modSF162	(2451)	2451	CCGCATCATCGAGGTGGCCAGCGCATCGGCCGCGCCTTCCTGCACATCC	2500
gp160.modSF162.delV2	(2370)		CCGCATCATCGAGGTGGCCAGCGCATCGGCCGCGCCTTCCTGCACATCC	
gp160.modSF162.delV1V2	(2262)		CCGCATCATCGAGGTGGCCAGCGCATCGGCCGCGCCTTCCTGCACATCC	
gp140.modSF162	(2026)		-----	
gp140.mut.modSF162	(2026)		-----	
gp140.mut7.modSF162	(2026)		-----	
gp140.mut8.modSF162	(2026)		-----	
gp120.modSF162	(1513)		-----	
Consensus	(2451)		-----	
gp160.modSF162	(2501)	2501	CCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAAC TCGAG	2547
gp160.modSF162.delV2	(2420)		CCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAAC TCGAG	
gp160.modSF162.delV1V2	(2312)		CCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAAC TCGAG	
gp140.modSF162	(2026)		-----	
gp140.mut.modSF162	(2026)		-----	
gp140.mut7.modSF162	(2026)		-----	
gp140.mut8.modSF162	(2026)		-----	
gp120.modSF162	(1513)		-----	
Consensus	(2501)		-----	

FIG. 66A-18

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		1	Start of tPA	40
gp160	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp160 del V1	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp160 del V2	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp160 del V1-2	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp 160 del 128-194	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp140TM	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp140	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp140mut	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
gp120	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
Consensus	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCT		
		41		80
gp160	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp160 del V1	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp160 del V2	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp160 del V1-2	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp 160 del 128-194	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp140TM	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp140	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp140mut	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp120	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
Consensus	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
		81	end of tPA	120
gp160	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp160 del V1	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp160 del V2	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp160 del V1-2	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp 160 del 128-194	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp140TM	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp140	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp140mut	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
gp120	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
Consensus	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTG		
		121		160
gp 160	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp160 del V1	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp160 del V2	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp160 del V1-2	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp 160 del 128-194	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp140TM	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp140	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp140mut	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
gp120	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		
Consensus	(121)	CCCGTGTTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA		

FIG. 66B-1

		98 / 131	200
		161	200
gp160	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp160 del V1	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp160 del V2	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp160 del V1-2	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp 160 del 128-194	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp140TM	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp140	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp140mut	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
gp120	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
Consensus	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAACGTGTG	
		201	240
gp160	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp160 del V1	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp160 del V2	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp160 del V1-2	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp 160 del 128-194	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp140TM	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp140	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp140mut	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
gp120	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
Consensus	(201)	GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAG	
		241	280
gp160	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp160 del V1	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp160 del V2	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp160 del V1-2	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp 160 del 128-194	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp140TM	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp140	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp140mut	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp120	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
Consensus	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
		281	320
gp160	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp160 del V1	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp160 del V2	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp160 del V1-2	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp 160 del 128-194	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp140TM	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp140	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp140mut	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp120	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
Consensus	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
		321	360
gp160	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp160 del V1	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp160 del V2	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp160 del V1-2	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp 160 del 128-194	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp140TM	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp140	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp140mut	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp120	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
Consensus	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	

FIG. 66B-2

		99/131	400
		361	
gp160	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA	
gp160 del V1	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGG	
gp160 del V2	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA	
gp160 del V1-2	(361)	GGC-----	
gp 160 del 128-194	(361)	ACCCCCCTGTGCGTGAGGGGAGGG-----	
gp140TM	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA	
gp140	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA	
gp140mut	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA	
gp120	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA	
Consensus	(361)	ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA	
		401	440
gp160	(401)	CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA	
gp160 del V1	(401)	GCGCCGGC-----	
gp160 del V2	(401)	CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(401)	CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA	
gp140	(401)	CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA	
gp140mut	(401)	CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA	
gp120	(401)	CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA	
Consensus	(401)	CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA	
		441	480
gp160	(441)	CAGCACCAGCGGCACCAACAGCACCAGCACCACCAACAGCACC	
gp160 del V1	(409)	-----	
gp160 del V2	(441)	CAGCACCAGCGGCACCAACAGCACCAGCACCACCAACAGCACC	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(441)	CAGCACCAGCGGCACCAACAGCACCAGCACCACCAACAGCACC	
gp140	(441)	CAGCACCAGCGGCACCAACAGCACCAGCACCACCAACAGCACC	
gp140mut	(441)	CAGCACCAGCGGCACCAACAGCACCAGCACCACCAACAGCACC	
gp120	(441)	CAGCACCAGCGGCACCAACAGCACCAGCACCACCAACAGCACC	
Consensus	(441)	CAGCACCAGCGGCACCAACAGCACCAGCACCACCAACAGCACC	
		481	520
gp160	(481)	GACAGCTGGGAGAAGATGCCCCGAGGGGCGAGATCAAGAACT	
gp160 del V1	(409)	-----GGCGAGATCAAGAACT	
gp160 del V2	(481)	GACAGCTGGGAGAAGATGCCCCGAGGGGCGAGATCAAGAACT	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(481)	GACAGCTGGGAGAAGATGCCCCGAGGGGCGAGATCAAGAACT	
gp140	(481)	GACAGCTGGGAGAAGATGCCCCGAGGGGCGAGATCAAGAACT	
gp140mut	(481)	GACAGCTGGGAGAAGATGCCCCGAGGGGCGAGATCAAGAACT	
gp120	(481)	GACAGCTGGGAGAAGATGCCCCGAGGGGCGAGATCAAGAACT	
Consensus	(481)	GACAGCTGGGAGAAGATGCCCCGAGGGGCGAGATCAAGAACT	
		521	560
gp160	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp160 del V1	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp160 del V2	(521)	GCAGCTTCAACATCGGCGCCGGC-----	
gp160 del V1-2	(521)	-----	
gp 160 del 128-194	(521)	-----	
gp140TM	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp140	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp140mut	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp120	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
Consensus	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	

FIG. 66B-3

100 / 131		561	600
gp160	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC	
gp160 del V1	(465)	GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC	
gp160 del V2	(544)	-----	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC	
gp140	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC	
gp140mut	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC	
gp120	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC	
Consensus	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC	
		601	640
gp160	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp160 del V1	(505)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp160 del V2	(544)	-----CGCCTGATCAACTGCA	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----AACTGCG	
gp140TM	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp140	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp140mut	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp120	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
Consensus	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
		641	680
gp160	(641)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
gp160 del V1	(545)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
gp160 del V2	(560)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
gp160 del V1-2	(364)	-----CAGGCCTGCCCCAAGGTGAGCTT	
gp 160 del 128-194	(392)	AGACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
gp140TM	(641)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
gp140	(641)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
gp140mut	(641)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
gp120	(641)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
Consensus	(641)	ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT	
		681	720
gp160	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp160 del V1	(585)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp160 del V2	(600)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp160 del V1-2	(387)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp 160 del 128-194	(432)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp140TM	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp140	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp140mut	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
gp120	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
Consensus	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC	
		721	760
gp160	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp160 del V1	(625)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp160 del V2	(640)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp160 del V1-2	(427)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp 160 del 128-194	(472)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp140TM	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp140	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp140mut	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp120	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
Consensus	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	

FIG. 66B-4

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		761	800
gp160	(761)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp160 del V1	(665)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp160 del V2	(680)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp160 del V1-2	(467)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp 160 del 128-194	(512)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp140TM	(761)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp140	(761)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp140mut	(761)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
gp120	(761)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
Consensus	(761)	GCCCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGG	
		801	840
gp160	(801)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp160 del V1	(705)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp160 del V2	(720)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp160 del V1-2	(507)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp 160 del 128-194	(552)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp140TM	(801)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp140	(801)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp140mut	(801)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp120	(801)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
Consensus	(801)	CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
		841	880
gp160	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp160 del V1	(745)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp160 del V2	(760)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp160 del V1-2	(547)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp 160 del 128-194	(592)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp140TM	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp140	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp140mut	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp120	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
Consensus	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
		881	920
gp160	(881)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp160 del V1	(785)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp160 del V2	(800)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp160 del V1-2	(587)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp 160 del 128-194	(632)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp140TM	(881)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp140	(881)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp140mut	(881)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
gp120	(881)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
Consensus	(881)	TCACCGACAACGCCAAGACCATCATCGTGCGCTGAGCTGAACGA	
		921	960
gp160	(921)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp160 del V1	(825)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp160 del V2	(840)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp160 del V1-2	(627)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp 160 del 128-194	(672)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp140TM	(921)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp140	(921)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp140mut	(921)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
gp120	(921)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	
Consensus	(921)	GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG	

FIG. 66B-5

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		961	1000
gp160	(961)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp160 del V1	(865)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp160 del V2	(880)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp160 del V1-2	(667)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp 160 del 128-194	(712)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp140TM	(961)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp140	(961)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp140mut	(961)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
gp120	(961)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
Consensus	(961)	CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG	
		1001	1040
gp160	(1001)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp160 del V1	(905)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp160 del V2	(920)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp160 del V1-2	(707)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp 160 del 128-194	(752)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp140TM	(1001)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp140	(1001)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp140mut	(1001)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
gp120	(1001)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
Consensus	(1001)	CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTG	
		1041	1080
gp160	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp160 del V1	(945)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp160 del V2	(960)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp160 del V1-2	(747)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp 160 del 128-194	(792)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp140TM	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp140	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp140mut	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp120	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
Consensus	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
		1081	1120
gp160	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp160 del V1	(985)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp160 del V2	(1000)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp160 del V1-2	(787)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp 160 del 128-194	(832)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp140TM	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp140	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp140mut	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
gp120	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
Consensus	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA	
		1121	1160
gp160	(1121)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp160 del V1	(1025)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp160 del V2	(1040)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp160 del V1-2	(827)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp 160 del 128-194	(872)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp140TM	(1121)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp140	(1121)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp140mut	(1121)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
gp120	(1121)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	
Consensus	(1121)	CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT	

FIG. 66B-6

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			1161	1200
gp160	(1161)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp160 del V1	(1065)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp160 del V2	(1080)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp160 del V1-2	(867)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp 160 del 128-194	(912)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp140TM	(1161)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp140	(1161)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp140mut	(1161)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
gp120	(1161)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
Consensus	(1161)	CGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC		
			1201	1240
gp160	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp160 del V1	(1105)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp160 del V2	(1120)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp160 del V1-2	(907)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp 160 del 128-194	(952)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp140TM	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp140	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp140mut	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
gp120	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
Consensus	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA		
			1241	1280
gp160	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp160 del V1	(1145)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp160 del V2	(1160)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp160 del V1-2	(947)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp 160 del 128-194	(992)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp140TM	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp140	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp140mut	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
gp120	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
Consensus	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT		
			1281	1320
gp160	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp160 del V1	(1185)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp160 del V2	(1200)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp160 del V1-2	(987)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp 160 del 128-194	(1032)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp140TM	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp140	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp140mut	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
gp120	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
Consensus	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG		
			1321	1360
gp160	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp160 del V1	(1225)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp160 del V2	(1240)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp160 del V1-2	(1027)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp 160 del 128-194	(1072)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp140TM	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp140	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp140mut	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
gp120	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		
Consensus	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC		

FIG. 66B-7

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		1361	1400
gp160	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp160 del V1	(1265)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp160 del V2	(1280)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp160 del V1-2	(1067)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp 160 del 128-194	(1112)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp140TM	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp140	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp140mut	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp120	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
Consensus	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
		1401	1440
gp160	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp160 del V1	(1305)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp160 del V2	(1320)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp160 del V1-2	(1107)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp 160 del 128-194	(1152)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp140TM	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp140	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp140mut	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp120	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
Consensus	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
		1441	1480
gp160	(1441)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp160 del V1	(1345)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp160 del V2	(1360)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp160 del V1-2	(1147)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp 160 del 128-194	(1192)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp140TM	(1441)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp140	(1441)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp140mut	(1441)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
gp120	(1441)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
Consensus	(1441)	GAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAAC	
		1481	1520
gp160	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp160 del V1	(1385)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp160 del V2	(1400)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp160 del V1-2	(1187)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp 160 del 128-194	(1232)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp140TM	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp140	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp140mut	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp120	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
Consensus	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
		1521	1560
gp160	(1521)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp160 del V1	(1425)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp160 del V2	(1440)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp160 del V1-2	(1227)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp 160 del 128-194	(1272)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp140TM	(1521)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp140	(1521)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp140mut	(1521)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
gp120	(1521)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	
Consensus	(1521)	GCCCCCTGGGCGTGGCCCCCAGGCAAGCGCCGCGTG	

FIG. 66B-8

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		1561	1600
gp160	(1561)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
gp160 del V1	(1465)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
gp160 del V2	(1480)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
gp160 del V1-2	(1267)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
gp 160 del 128-194	(1312)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
gp140TM	(1561)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
gp140	(1561)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
gp140mut	(1561)	GTGCAGCGCGAGAAGAGCGCCGTGGGCCTGGGCGCCCTGT	
gp120	(1561)	GTGCAGCGCGAGAAGCGCTAAG-----	
Consensus	(1561)	GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT	
		1601	1640
gp160	(1601)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp160 del V1	(1505)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp160 del V2	(1520)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp160 del V1-2	(1307)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp 160 del 128-194	(1352)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp140TM	(1601)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp140	(1601)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp140mut	(1601)	TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG	
gp120	(1583)	ATATCGGATCCTCTAGA-----	
Consensus	(1601)	TCATCGGCTTCNCTGGGCGCCGCCGGGAGCACCATGGGCG	
		1641	1680
gp160	(1640)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp160 del V1	(1544)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp160 del V2	(1559)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp160 del V1-2	(1346)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp 160 del 128-194	(1391)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp140TM	(1640)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp140	(1640)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp140mut	(1640)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
gp120	(1600)	-----	
Consensus	(1641)	CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT	
		1681	1720
gp160	(1680)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp160 del V1	(1584)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp160 del V2	(1599)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp160 del V1-2	(1386)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp 160 del 128-194	(1431)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp140TM	(1680)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp140	(1680)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp140mut	(1680)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
gp120	(1600)	-----	
Consensus	(1681)	GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC	
		1721	1760
gp160	(1720)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp160 del V1	(1624)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp160 del V2	(1639)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp160 del V1-2	(1426)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp 160 del 128-194	(1471)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp140TM	(1720)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp140	(1720)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp140mut	(1720)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	
gp120	(1600)	-----	
Consensus	(1721)	ATCGAGGCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG	

FIG. 66B-9

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Date: 06 jul 2000

Destination: Agent

Address:

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		1761	1800
gp160	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp160 del V1	(1664)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp160 del V2	(1679)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp160 del V1-2	(1466)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp 160 del 128-194	(1511)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp140TM	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp140	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp140mut	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp120	(1600)	-----	
Consensus	(1761)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
		1801	1840
gp160	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp160 del V1	(1704)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp160 del V2	(1719)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp160 del V1-2	(1506)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp 160 del 128-194	(1551)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp140TM	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp140	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp140mut	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp120	(1600)	-----	
Consensus	(1801)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
		1841	1880
gp160	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp160 del V1	(1744)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp160 del V2	(1759)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp160 del V1-2	(1546)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp 160 del 128-194	(1591)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp140TM	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp140	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp140mut	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp120	(1600)	-----	
Consensus	(1841)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
		1881	1920
gp160	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp160 del V1	(1784)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp160 del V2	(1799)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp160 del V1-2	(1586)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp 160 del 128-194	(1631)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp140TM	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp140	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp140mut	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp120	(1600)	-----	
Consensus	(1881)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
		1921	1960
gp160	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp160 del V1	(1824)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp160 del V2	(1839)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp160 del V1-2	(1626)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp 160 del 128-194	(1671)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp140TM	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp140	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp140mut	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp120	(1600)	-----	
Consensus	(1921)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	

FIG. 66B-10

SUBSTITUTE SHEET (RULE 26)

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		1961	2000
gp160	(1960)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp160 del V1	(1864)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp160 del V2	(1879)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp160 del V1-2	(1666)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp 160 del 128-194	(1711)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp140TM	(1960)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp140	(1960)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp140mut	(1960)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
gp120	(1600)	-----	
Consensus	(1961)	ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC	
		2001	2040
gp160	(2000)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp160 del V1	(1904)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp160 del V2	(1919)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp160 del V1-2	(1706)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp 160 del 128-194	(1751)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp140TM	(2000)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp140	(2000)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp140mut	(2000)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
gp120	(1600)	-----	
Consensus	(2001)	AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA	
		2041	2080
gp160	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp160 del V1	(1944)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp160 del V2	(1959)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp160 del V1-2	(1746)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp 160 del 128-194	(1791)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp140TM	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp140	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp140mut	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
gp120	(1600)	-----	
Consensus	(2041)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGG	
		2081	2120
gp160	(2080)	CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC	
gp160 del V1	(1984)	CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC	
gp160 del V2	(1999)	CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC	
gp160 del V1-2	(1786)	CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC	
gp 160 del 128-194	(1831)	CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC	
gp140TM	(2080)	CTGTGGTACATC-----	
gp140	(2080)	CTGTGGTACATC-----	
gp140mut	(2080)	CTGTGGTACATC-----	
gp120	(1600)	-----	
Consensus	(2081)	CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC	
		2121	2160
gp160	(2120)	TGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCA----	
gp160 del V1	(2024)	TGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCA----	
gp160 del V2	(2039)	TGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCA----	
gp160 del V1-2	(1826)	TGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCA----	
gp 160 del 128-194	(1871)	TGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCA----	
gp140TM	(2120)	TGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCATCGT	
gp140	(2092)	-----	
gp140mut	(2092)	-----	
gp120	(1600)	-----	
Consensus	(2121)	TGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCANNNN	

FIG. 66B-11

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		2161	2200
gp160	(2156)	-TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC	
gp160 del V1	(2060)	-TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC	
gp160 del V2	(2075)	-TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC	
gp160 del V1-2	(1862)	-TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC	
gp 160 del 128-194	(1907)	-TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC	
gp140TM	(2160)	GTAAGATATCGGATCCTCTAGA-----	
gp140	(2092)	-TAAGATATCGGATCCTCTAGA-----	
gp140mut	(2092)	-TAAGATATCGGATCCTCTAGA-----	
gp120	(1600)	-----	
Consensus	(2161)	NTCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC	
		2201	2240
gp160	(2195)	TGCAGACCCGCCTGCCCCGCCAGCGCGGCCCCGACCGCCC	
gp160 del V1	(2099)	TGCAGACCCGCCTGCCCCGCCAGCGCGGCCCCGACCGCCC	
gp160 del V2	(2114)	TGCAGACCCGCCTGCCCCGCCAGCGCGGCCCCGACCGCCC	
gp160 del V1-2	(1901)	TGCAGACCCGCCTGCCCCGCCAGCGCGGCCCCGACCGCCC	
gp 160 del 128-194	(1946)	TGCAGACCCGCCTGCCCCGCCAGCGCGGCCCCGACCGCCC	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2201)	TGCAGACCCGCCTGCCCCGCCAGCGCGGCCCCGACCGCCC	
		2241	2280
gp160	(2235)	CGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC	
gp160 del V1	(2139)	CGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC	
gp160 del V2	(2154)	CGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC	
gp160 del V1-2	(1941)	CGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC	
gp 160 del 128-194	(1986)	CGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2241)	CGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC	
		2281	2320
gp160	(2275)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp160 del V1	(2179)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp160 del V2	(2194)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp160 del V1-2	(1981)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp 160 del 128-194	(2026)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2281)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
		2321	2360
gp160	(2315)	GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG	
gp160 del V1	(2219)	GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG	
gp160 del V2	(2234)	GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG	
gp160 del V1-2	(2021)	GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG	
gp 160 del 128-194	(2066)	GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2321)	GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG	

FIG. 66B-12

SUBSTITUTE SHEET (RULE 26)

		109/131	
		2361	2400
gp160	(2355)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp160 del V1	(2259)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp160 del V2	(2274)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp160 del V1-2	(2061)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp 160 del 128-194	(2106)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2361)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
		2401	2440
gp160	(2395)	CTGCTGGGCGCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp160 del V1	(2299)	CTGCTGGGCGCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp160 del V2	(2314)	CTGCTGGGCGCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp160 del V1-2	(2101)	CTGCTGGGCGCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp 160 del 128-194	(2146)	CTGCTGGGCGCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2401)	CTGCTGGGCGCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
		2441	2480
gp160	(2435)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp160 del V1	(2339)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp160 del V2	(2354)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp160 del V1-2	(2141)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp 160 del 128-194	(2186)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2441)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
		2481	2520
gp160	(2475)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp160 del V1	(2379)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp160 del V2	(2394)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp160 del V1-2	(2181)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp 160 del 128-194	(2226)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2481)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
		2521	2560
gp160	(2515)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp160 del V1	(2419)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp160 del V2	(2434)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp160 del V1-2	(2221)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp 160 del 128-194	(2266)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2521)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	

FIG. 66B-13

SUBSTITUTE SHEET (RULE 26)

[illegible]

FIG. 66B-14

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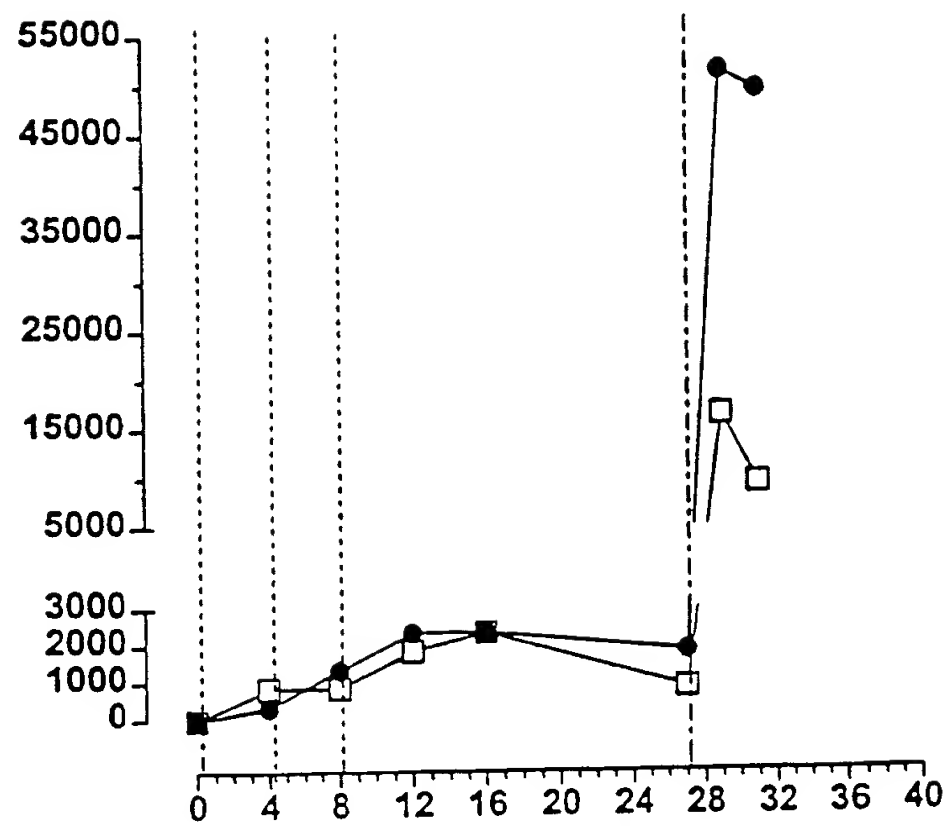


FIG. 67

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HIV-1SF2 wt RT (PISPIET-->GIRKVL)
CCCATTAGTCCTATTGAAACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCCAAAA
GTTAAGCAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAGATATGTACAGAA
ATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTA
TTTGCTATAAAGAAAAAAGACAGTACTAAATGGAGAAAAGTAGTAGATTTTCAGAGAACTT
AATAAAAGAACTCAAGACTTCTGGGAAGTTTTCAGTTAGGAATACCACACCCCGCAGGGTTA
AAAAAGAAAAAATCAGTAACAGTATTGGATGTGGGTGATGCATACTTTTCAGTTCCCTTA
GATAAAGACTTTAGAAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGACACCA
GGGATTAGATATCAGTACAATGTGCTGCCACAGGGATGGAAAGGATCACCAGCAATATTC
CAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAACAGAATCCAGACATAGTTATC
TATCAAtacatggatgatTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACA
AAAATAGAGGAAGTGGAGACAGCATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAAA
CATCAGAAAGAACCTCCATTCCTTtggatgggttatGAACTCCATCCTGATAAATGGACA
GTACAGCCTATAATGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTA
GTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGGATTAAAGTAAAGCAGTTATGT
AACTCCTTAGAGGAACCAAGCACTAACAGAAGTAATACTACTAACAGAAGAAGCAGAG
CTAGAAGTGGCAGAAAACAGGGAGATTCTAAAAGAACCAGTACATGAAGTATATTATGAC
CCATCAAAAGACTTAGTAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAA
ATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGGATGAGGGGTGCC
CACACTAATGATGTAAAACAGTTAACAGAGGCAGTGCAAAAAGTATCCACAGAAAGCATA
GTAATATGGGGAAAGATTCTTAAATTTAACTACCCATACAAAAGGAAACATGGGAAGCA
TGGTGGATGGAGTATTGGCAAGCTACCTGGATTCTTGAGTGGGAGTTTGTCAATACCCCT
CCCTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCCATAGTAGGAGCAGAACTTTC
TATGTAGATGGGGCAGCTAATAGGGAGACTAAATTAGGAAAAGCAGGATATGTTACTGAC
AGAGGAAGACAAAAAGTTGTCTCCATAGCTGACACAACAAATCAGAAGACTGAATTACAA
GCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTAACAGACTCACAA
TATGCATTAGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAA
ATAATAGAGCAGTTAATAAAAAAGGAAAAGGTCTACCTGGCATGGGTACCAGCACACAAA
GGAATTGGAGGAAATGAACAAGTAGATAAATTAGTCAGTGCTGGAATCAGGAAAGTACTA

FIG. 68

(SEQ ID NO:77)

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GagProtMod.SF2 (GP1)

GTTCGACGCCACCATGGGCGCCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGG
GAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGG
GCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGC
TGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGC
AGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGAC
ACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAAGTCCAAGAAGAAGGCCAG
CAGGCCGCGCGCCGCGCCGGCACC GGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATC
GTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCC
TGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCC
CTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCAC
CAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCGC
GTGCACCCCGTGCACGCGCGGCCCATCGCCCCGGCCAGATGCGCGAGCCCCGCGGCAGC
GACATCGCCGGCACCACCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCC
CCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTG
CGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGC
GACTACGTGGACCGCTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAG
AACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTG
AAGGCTCTCGGCCCCGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGC
GGCCCCGGCCACAAGGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCG
ACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAAC
TGCGGCAAGGAGGGCCACACCGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGG
CGCTGCGGCCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTA
GGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAG
CCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACCTCCCTCTCAG
AAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGC
AACGACCCCTCGTCACAGTAAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCG
GCGCCGACGACACCGTGCTGGAGGAGATGAACCTGCCCGGCAAGTGGAAGCCCAAGATGA
TCGGCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCT
GCGGCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCGTGAACATCATCGGCC
GCAACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGG
TGCCCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCTGTAAG
AATTC

FIG. 69

(SEQ ID NO:78)

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GagProtMod.SF2 (GP2)

GTTCGACGCCACCATGGGCGCCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGG
GAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGG
GCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGC
TGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGC
AGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGAC
ACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCCAG
CAGGCCGCGCGCCGCCCGGCACCGGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATC
GTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCC
TGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCC
CTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCAC
CAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCGCGGAGTGGGACCGC
GTGCACCCCGTGCACGCCCGGCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCAGC
GACATCGCCGGCACCACCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCC
CCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTG
CGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGC
GACTACGTGGACCGCTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAG
AACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTG
AAGGCTCTCGGCCCCGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGC
GGCCCCGGCCACAAGGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCG
ACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAAC
TGCGGCAAGGAGGGCCACACCGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGG
CGCTGCGGCGCGGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTA
GGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAG
CCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACCTCCCTCTCAG
AAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGC
AACGACCCCTCGTCACAGTAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAG
GAGCAGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAAACCAAAAATGA
TAGGGGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCT
GTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAA
GAAATCTGTTGACCCAGATCGGCTGCACCTTGAACCTCCCCATCAGCCCTATTGAGACGG
TGCCCGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGTAAG
AATTC

FIG. 70

(SEQ ID NO:79)

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FS(+)_ProtInact_RTopt_YM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTTAGGGA
AGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAA
CAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACTCCCTCTCAGAAGC
AGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACG
ACCCCTCGTCACAATAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAGGAGC
AGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAAACCAAAAATGATAGG
GGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCTGTGG
ACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAA
TCTGTTGACCCAGATCGGCTGCACCTTGAACCTCCCCATCAGCCCTATTGAGACGGTGCC
CGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGACCGAGGA
GAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCAA
GATCGGGCCCCGAGAACCCCTACAACACCCCCGTGTTCCGCCATCAAGAAGAAGGACAGCAC
CAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGA
GGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGCT
GGACGTGGGCGACGCCTACTTCAGCGTGCCCCCTGGACAAGGACTTCCGCAAGTACACCGC
CTTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAACGTGCT
GCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGGA
GCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCAG
CGACCTGGAGATCGGCCAGCACCCGACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGCG
CTGGGGCTTCACCACCCCGACAAGAAGCACCAAGGAGCCCCCCTTCTGTGGATGGG
CTACGAGCTGCACCCCGACAAGTGGACCGTGCAGCCCATCATGCTGCCCCGAGAAGGACAG
CTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTA
CGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGA
GGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAA
GGAGCCCGTGCACGAGGTGTACTACGACCCCGCAAGGACCTGGTGGCCGAGATCCAGAA
GCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGAC
CGGCAAGTACGCCCCGATGCGCGGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGC
CGTGCAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCT

FIG. 71A

(SEQ ID NO:80)

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CCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGATCCCCGA
GTGGGAGTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCC
CATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCGAGACCAAGCTGGG
CAAGGCCGGCTACGTGACCGACCGGGGGCCGGCAGAAGGTGGTGAGCATCGCCGACACCAC
CAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCTGGAGGT
GAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCCAGCCCGACAAGAG
CGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCT
GGCCTGGGTGCCCCGCCCAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCTGGTGAG
CGCCGGCATCCGCAAGGTGCTGTTCTTGAACGGCATCGATGGCGGCATCGTGATCTACCA
GTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCTTCCCGG
GGCTAGCACCGGTGAATTC

FIG. 72B

(SEQ ID NO:81)

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FS(-)_ProtMod_RTpt_YM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCC
AGGACCTGGCCTTCCTGCAGGGCAAGGCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCGGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCGTGAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCTGAACTTCCCCATCAGCCCCATCGAGACGGTG
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGCCCCGAGAACCCTACAACACCCCCGTGTTCCGCCATCAAGAAGAAGGACAGCA
CCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTG
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCA
GCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGC
GCTGGGGCTTCACCACCCCGACAAGAAGCACCAAGGAGCCCCCCTTCCTGTGGATGG
GCTACGAGCTGCACCCCGACAAGTGGACCGTGACGCCCATCATGCTGCCCCGAGAAGGACA
GCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCT
ACGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCG
AGGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGA
AGGAGCCCGTGACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGA
AGCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGA
CCGGCAAGTACGCCCGCATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGG
CCGTGCAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGC

FIG. 73A

(SEQ ID NO:82)

SUBSTITUTE SHEET (RULE 26)

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TGCCCCATCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGA
TCCCCGAGTGGGAGTTTCGTGAACACCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGA
AGGAGCCCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCGAGACCA
AGCTGGGCAAGGCCGGCTACGTGACCGACCGGGGCCGGCAGAAGGTGGTGAGCATCGCCG
ACACCACCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCC
TGGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCCCG
ACAAGAGCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGG
TGTACCTGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGC
TGGTGAGCGCCGGCATCCGCAAGGTGCTGTTCTGAACGGCATCGATGGCGGCATCGTGA
TCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGC
TTCCCGGGGCTAGCACCGGTGAATTC

FIG. 73B
(SEQ ID NO:82)

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FS(-)_ProtMod_RTpt_YMWM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCG
AGGACCTGGCCTTCCTGCAGGGCAAGGCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCCGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCCGTGAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTGC
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGCCCCGAGAACCCTACAACACCCCCGTGTTCCGCATCAAGAAGAAGGACAGCA
CCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGC
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCA
GCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGC
GCTGGGGCTTCACCACCCCGACAAGAAGCACCAAGGAGCCCCCCTTCTGCCCCATCG
AGCTGCACCCCGACAAGTGGACCGTGCGAGCCCATCATGCTGCCCCGAGAAGGACAGCTGGA
CCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTACGCCG
GCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGAGGTGA
TCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAAGGAGC
CCGTGCACGAGGTGTACTACGACCCCGACAAGGACCTGGTGGCCGAGATCCAGAAGCAGG
GCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCA
AGTACGCCCGCATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGC
AGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCTGCCCA

FIG. 74A

(SEQ ID NO:83)

TCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGATCCCCG
AGTGGGAGTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGC
CCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCGAGACCAAGCTGG
GCAAGGCCGGCTACGTGACCGACCGGGGGCCGGCAGAAGGTGGTGAGCATCGCCGACACCA
CCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCTGGAGG
TGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCCCAGCCCGACAAGA
GCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACC
TGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCTGGTGA
GCGCCGGCATCCGCAAGGTGCTGTTCTGAACGGCATCGATGGCGGCATCGTGATCTACC
AGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCTTCCCCG
GGGCTAGCACCGGTGAATTC

FIG. 74B

(SEQ ID NO:83)

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FS(-)_ProtMod_RTopt(+)

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCG
AGGACCTGGCCTTCCTGCAGGGCAAGGCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCCCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCGGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCGTGAAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTG
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGCCCCGAGAACCCCTACAACACCCCGTGTTCCGCCATCAAGAAGAAGGACAGCA
CCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGC
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGTACATGGACGACCTGTACG
TGGGCAGCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCC
TGCTGCGCTGGGGCTTCACCACCCCGACAAGAAGCACCAAGGAGCCCCCCTTCCTGT
GGATGGGCTACGAGCTGCACCCCGACAAGTGGACCGTGCGAGCCCATCATGCTGCCCCGAGA
AGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCC
AGATCTACGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCC
TGACCGAGGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGA
TCCTGAAGGAGCCCGTGACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGA
TCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACC
TGAAGACCGGCAAGTACGCCCCGCATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGA
CCGAGGCCGTGCAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGT
TCAAGCTGCCCATCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCA
CCTGGATCCCCGAGTGGGAGTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGC
TGGAGAAGGAGCCCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC

FIG. 75A

(SEQ ID NO:84)

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AGACCAAGCTGGGCAAGGCCGGCTACGTGACCGACCGGGGCCGGCAGAAGGTGGTGAGCA
TCGCCGACACCACCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACA
GCGGCCTGGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCC
AGCCCGACAAGAGCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGG
AGAAGGTGTACCTGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGG
ACAAGCTGGTGAGCGCCGGCATCCGCAAGGTGCTGTTCTGAACGGCATCGATGGCGGCA
TCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATT
AAAAGCTTCCCGGGGCTAGCACCGGTGAATTC

FIG. 75B

(SEQ ID NO:84)

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Tat_wt_SF162 (wildtype)

ATGGAGCCAGTAGATCCTAGATTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAGA
CTGCTTGTACAAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTCATAAC
AAAAGGCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCCT
CCAGACAGTGAGGTTTCATCAAGTTTCTCTACCAAAGCAACCCGCTTCCCAGCCCCAAGG
GGACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGA
TCCAGTCCATTAG

FIG. 76

(SEQ ID NO:85)

Tat_SF162

MEPVDPRLPEWKHPGSQPKTACTNICYCKKCCFHCQVCFITKGLGISYGRKKRRQRRRAPDSE
VHQVSLPKQPASQPQGDPTGPKESKKKVERETETDPVH

FIG. 77

(SEQ ID NO:86)

Tat_SF162_opt

ATGGAGCCCGTGGACCCCCGCCTGGAGCCCTGGAAGCACCCCGGCAGCCAGCCCAAGAC
CGCCTGCACCAACTGCTACTGCAAGAAGTGCTGCTTCCACTGCCAGGTGTGCTTCATCACC
AAGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGCGCCCCCCCCC
CGACAGCGAGGTGCACCAGGTGAGCCTGCCCAAGCAGCCCGCCAGCCAGCCCCAGGGCG
ACCCACCGGCCCCAAGGAGAGCAAGAAGAAGGTGGAGCGCGAGACCGAGACCGACCCC
GTGCACTAG

FIG. 78

(SEQ ID NO:87)

Tat_Cys22_SF162_opt

ATGGAGCCCGTGGACCCCCGCCTGGAGCCCTGGAAGCACCCCGGCAGCCAGCCCAAGAC
CGCCGACCAACTGCTACTGCAAGAAGTGCTGCTTCCACTGCCAGGTGTGCTTCATCACCA
AGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGCGCCCCCCCCC
GACAGCGAGGTGCACCAGGTGAGCCTGCCCAAGCAGCCCGCCAGCCAGCCCCAGGGCGA
CCCCACCGGCCCCAAGGAGAGCAAGAAGAAGGTGGAGCGCGAGACCGAGACCGACCCCG
TGCACTAG

FIG. 79

(SEQ ID NO:88)

	Section 1																																																			
	(1)	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500
GagMod.SF2	(1)	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500
GagProtMod.SF2 (GP1)	(1)	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500
GagProtMod.SF2 (GP2)	(1)	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500
Consensus	(1)	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500
GagMod.SF2	(77)	77	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500								
GagProtMod.SF2 (GP1)	(77)	77	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500								
GagProtMod.SF2 (GP2)	(77)	77	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500								
Consensus	(77)	77	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500								
GagMod.SF2	(153)	153	160																																																	

FIG. 80A

Alignment GagMod vs GP1 GP2

	(381)	381	390	400	410	420	430	440	456	Section 6
GagMod.SF2	(381)	CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGCAGGGCCAGATGGTGCAACGAGGCCATCAGCCCCCGC								
GagProtMod.SF2(GP1)	(381)	CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGCAGGGCCAGATGGTGCAACGAGGCCATCAGCCCCCGC								
GagProtMod.SF2(GP2)	(381)	CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGCAGGGCCAGATGGTGCAACGAGGCCATCAGCCCCCGC								
Consensus	(381)	CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGCAGGGCCAGATGGTGCAACGAGGCCATCAGCCCCCGC								Section 7
	(457)	457	470	480	490	500	510	520	532	
GagMod.SF2	(457)	ACCTGAACGCCCTGGTGAAGTGGTGAGGAGAAAGCCCTTCAGCCCCGAGGTGATCCCCCATGTTTCAGCGCCCTGA								
GagProtMod.SF2(GP1)	(457)	ACCTGAACGCCCTGGTGAAGTGGTGAGGAGAAAGCCCTTCAGCCCCGAGGTGATCCCCCATGTTTCAGCGCCCTGA								
GagProtMod.SF2(GP2)	(457)	ACCTGAACGCCCTGGTGAAGTGGTGAGGAGAAAGCCCTTCAGCCCCGAGGTGATCCCCCATGTTTCAGCGCCCTGA								
Consensus	(457)	ACCTGAACGCCCTGGTGAAGTGGTGAGGAGAAAGCCCTTCAGCCCCGAGGTGATCCCCCATGTTTCAGCGCCCTGA								Section 8
	(533)	533	540	550	560	570	580	590	608	
GagMod.SF2	(533)	GCGAGGGCGCCACCCCCCAGGACCTGAACACCGATGTTGAACACCCGTGGCGGCCACCAAGGCCGCCATGCAGATGCT								
GagProtMod.SF2(GP1)	(533)	GCGAGGGCGCCACCCCCCAGGACCTGAACACCGATGTTGAACACCCGTGGCGGCCACCAAGGCCGCCATGCAGATGCT								
GagProtMod.SF2(GP2)	(533)	GCGAGGGCGCCACCCCCCAGGACCTGAACACCGATGTTGAACACCCGTGGCGGCCACCAAGGCCGCCATGCAGATGCT								
Consensus	(533)	GCGAGGGCGCCACCCCCCAGGACCTGAACACCGATGTTGAACACCCGTGGCGGCCACCAAGGCCGCCATGCAGATGCT								Section 9
	(609)	609	620	630	640	650	660	670	684	
GagMod.SF2	(609)	GAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCCGCTGCACCCCGTGCAAGCCGCCCATCGCCCCCGC								
GagProtMod.SF2(GP1)	(609)	GAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCCGCTGCACCCCGTGCAAGCCGCCCATCGCCCCCGC								
GagProtMod.SF2(GP2)	(609)	GAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCCGCTGCACCCCGTGCAAGCCGCCCATCGCCCCCGC								
Consensus	(609)	GAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCCGCTGCACCCCGTGCAAGCCGCCCATCGCCCCCGC								Section 10
	(685)	685	690	700	710	720	730	740	750	760
GagMod.SF2	(685)	CAGATGCGCGAGCCCCCGGGCAGCGACATCGCCGGCACCAACCAAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA								
GagProtMod.SF2(GP1)	(685)	CAGATGCGCGAGCCCCCGGGCAGCGACATCGCCGGCACCAACCAAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA								
GagProtMod.SF2(GP2)	(685)	CAGATGCGCGAGCCCCCGGGCAGCGACATCGCCGGCACCAACCAAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA								
Consensus	(685)	CAGATGCGCGAGCCCCCGGGCAGCGACATCGCCGGCACCAACCAAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA								

FIG. 80B

Alignment GagMod vs GP1_GP2

	(761)	761	770	780	790	800	810	820	Section 11
GagMod.SF2	(761)	ACAACCC	CCCCAT	CCCCGT	GGCGGAGAT	CTACAAG	CGGTGGAT	CATCCT	GGCCCTGAACAAGATCGTGGCGGATGTA
GagProtMod.SF2(GP1)	(761)	ACAACCC	CCCCAT	CCCCGT	GGCGGAGAT	CTACAAG	CGGTGGAT	CATCCT	GGCCCTGAACAAGATCGTGGCGGATGTA
GagProtMod.SF2(GP2)	(761)	ACAACCC	CCCCAT	CCCCGT	GGCGGAGAT	CTACAAG	CGGTGGAT	CATCCT	GGCCCTGAACAAGATCGTGGCGGATGTA
Consensus	(761)	ACAACCC	CCCCAT	CCCCGT	GGCGGAGAT	CTACAAG	CGGTGGAT	CATCCT	GGCCCTGAACAAGATCGTGGCGGATGTA
									Section 12
	(837)	837	850	860	870	880	890	900	912
GagMod.SF2	(837)	CAGCCCC	ACACGAT	CCTGGACAT	CCGCCAGG	CCCCCAAG	GAGCCCTT	CCGCGACT	ACGTGGACCGCTTCTACAAG
GagProtMod.SF2(GP1)	(837)	CAGCCCC	ACACGAT	CCTGGACAT	CCGCCAGG	CCCCCAAG	GAGCCCTT	CCGCGACT	ACGTGGACCGCTTCTACAAG
GagProtMod.SF2(GP2)	(837)	CAGCCCC	ACACGAT	CCTGGACAT	CCGCCAGG	CCCCCAAG	GAGCCCTT	CCGCGACT	ACGTGGACCGCTTCTACAAG
Consensus	(837)	CAGCCCC	ACACGAT	CCTGGACAT	CCGCCAGG	CCCCCAAG	GAGCCCTT	CCGCGACT	ACGTGGACCGCTTCTACAAG
									Section 13
	(913)	913	920	930	940	950	960	970	988
GagMod.SF2	(913)	ACCCTG	CGCGCT	GAGCAGG	CCAGCCAGG	ACCTGAAGA	ACTGGAT	GACCGAG	ACCCCTGCTGGTGAGAACGCCAACCC
GagProtMod.SF2(GP1)	(913)	ACCCTG	CGCGCT	GAGCAGG	CCAGCCAGG	ACCTGAAGA	ACTGGAT	GACCGAG	ACCCCTGCTGGTGAGAACGCCAACCC
GagProtMod.SF2(GP2)	(913)	ACCCTG	CGCGCT	GAGCAGG	CCAGCCAGG	ACCTGAAGA	ACTGGAT	GACCGAG	ACCCCTGCTGGTGAGAACGCCAACCC
Consensus	(913)	ACCCTG	CGCGCT	GAGCAGG	CCAGCCAGG	ACCTGAAGA	ACTGGAT	GACCGAG	ACCCCTGCTGGTGAGAACGCCAACCC
									Section 14
	(989)	989	1000	1010	1020	1030	1040	1050	1064
GagMod.SF2	(989)	CCGACT	GCAAGAC	CAATCCT	GAAGGCT	CTCGGCC	CCCGCGCC	ACCCCTG	GAGGAGATGATGACCGCCTGCCAGGGCGT
GagProtMod.SF2(GP1)	(989)	CCGACT	GCAAGAC	CAATCCT	GAAGGCT	CTCGGCC	CCCGCGCC	ACCCCTG	GAGGAGATGATGACCGCCTGCCAGGGCGT
GagProtMod.SF2(GP2)	(989)	CCGACT	GCAAGAC	CAATCCT	GAAGGCT	CTCGGCC	CCCGCGCC	ACCCCTG	GAGGAGATGATGACCGCCTGCCAGGGCGT
Consensus	(989)	CCGACT	GCAAGAC	CAATCCT	GAAGGCT	CTCGGCC	CCCGCGCC	ACCCCTG	GAGGAGATGATGACCGCCTGCCAGGGCGT
									Section 15
	(1065)	1065	1070	1080	1090	1100	1110	1120	1140
GagMod.SF2	(1065)	GGCGG	CCCCCG	CCACAA	GGCCCG	CGTGTG	CGCGAGG	CGATG	AGCCAGGTGACGAAACCCGGCGGACCATCATGATG
GagProtMod.SF2(GP1)	(1065)	GGCGG	CCCCCG	CCACAA	GGCCCG	CGTGTG	CGCGAGG	CGATG	AGCCAGGTGACGAAACCCGGCGGACCATCATGATG
GagProtMod.SF2(GP2)	(1065)	GGCGG	CCCCCG	CCACAA	GGCCCG	CGTGTG	CGCGAGG	CGATG	AGCCAGGTGACGAAACCCGGCGGACCATCATGATG
Consensus	(1065)	GGCGG	CCCCCG	CCACAA	GGCCCG	CGTGTG	CGCGAGG	CGATG	AGCCAGGTGACGAAACCCGGCGGACCATCATGATG

FIG. 80C

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Alignment GagMod vs GP1_GP2

	1141	1150	1160	1170	1180	1190	1200	Section 16
GagMod.SF2(1141)	(1141)	1150	1160	1170	1180	1190	1200	1216
GagProtMod.SF2(GP1)(1141)	CAGCGGCGCAACTTCCGCAACACGCGGAGACCGTCAAGTGTCTCAACTGCGGCAAGGAGGCGCCACACCGCCAGGA							
GagProtMod.SF2(GP2)(1141)	CAGCGGCGCAACTTCCGCAACACGCGGAGACCGTCAAGTGTCTCAACTGCGGCAAGGAGGCGCCACACCGCCAGGA							
Consensus(1141)	CAGCGGCGCAACTTCCGCAACACGCGGAGACCGTCAAGTGTCTCAACTGCGGCAAGGAGGCGCCACACCGCCAGGA							
		1230	1240	1250	1260	1270	1280	Section 17
GagMod.SF2(1217)	(1217)	1230	1240	1250	1260	1270	1280	1292
GagProtMod.SF2(GP1)(1217)	ACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGGAGGCGCCACCAAGATGAAGGACTGCACCGAGCG							
GagProtMod.SF2(GP2)(1217)	ACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGGAGGCGCCACCAAGATGAAGGACTGCACCGAGCG							
Consensus(1217)	ACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGGAGGCGCCACCAAGATGAAGGACTGCACCGAGCG							
		1310	1320	1330	1340	1350		Section 18
GagMod.SF2(1293)	(1293)	1310	1320	1330	1340	1350		1368
GagProtMod.SF2(GP1)(1293)	CCAGGCCAACTTCTTGGGCAAGATCTGGCCAGCTACAAAGGCGCGCCCGGCAACTTCTTCAGAGAGCGCCCGGAG							
GagProtMod.SF2(GP2)(1293)	CCAGGCCAACTTCTTGGGCAAGATCTGGCCAGCTACAAAGGCGCGCCCGGCAACTTCTTCAGAGAGCGCCCGGAG							
Consensus(1293)	CCAGGCCAACTTCTTGGGCAAGATCTGGCCAGCTACAAAGGCGCGCCCGGCAACTTCTTCAGAGAGCGCCCGGAG							
		1380	1390	1400	1410	1420	1430	Section 19
GagMod.SF2(1369)	(1369)	1380	1390	1400	1410	1420	1430	1444
GagProtMod.SF2(GP1)(1369)	CCACACGCGCCCCCGGAGAGAGCTTCCGCTTCCGCGAGGAGAGAACCCACCCCGGCAAGAGCAGGAGCGCCATCG							
GagProtMod.SF2(GP2)(1369)	CCACACGCGCCCCCGGAGAGAGCTTCCGCTTCCGCGAGGAGAGAACCCACCCCGGCAAGAGCAGGAGCGCCATCG							
Consensus(1369)	CCACACGCGCCCCCGGAGAGAGCTTCCGCTTCCGCGAGGAGAGAACCCACCCCGGCAAGAGCAGGAGCGCCATCG							
		1460	1470	1480	1490	1500	1510	Section 20
GagMod.SF2(1445)	(1445)	1460	1470	1480	1490	1500	1510	1520
GagProtMod.SF2(GP1)(1445)	ACAAGGAGCTGTATCCCTTAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTACAGCCAGTAA-----							
GagProtMod.SF2(GP2)(1445)	ACAAGGAGCTGTATCCCTTAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTACAGCCAGTAA-----							
Consensus(1445)	ACAAGGAGCTGTATCCCTTAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTACAGCCAGTAA-----							

FIG. 80D

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Alignment GagMod vs GP1_GP2

	1521	1530	1540	1550	1560	1570	1580	1596	Section 21
GagMod.SF2(1510)	(1521)	1521	1530	1540	1550	1560	1570	1580	1596
GagProtMod.SF2(GP1)(1521)									
GagProtMod.SF2(GP2)(1521)									
Consensus(1521)									
GagMod.SF2(1510)	(1597)	1597	1610	1620	1630	1640	1650	1660	1672
GagProtMod.SF2(GP1)(1597)									
GagProtMod.SF2(GP2)(1597)									
Consensus(1597)									
GagMod.SF2(1510)	(1673)	1673	1680	1690	1700	1710	1720	1730	1748
GagProtMod.SF2(GP1)(1673)									
GagProtMod.SF2(GP2)(1673)									
Consensus(1673)									
GagMod.SF2(1510)	(1749)	1749	1760	1770	1780	1790	1800	1810	1824
GagProtMod.SF2(GP1)(1749)									
GagProtMod.SF2(GP2)(1749)									
Consensus(1749)									
GagMod.SF2(1510)	(1825)	1825	1830	1847					
GagProtMod.SF2(GP1)(1825)									
GagProtMod.SF2(GP2)(1825)									
Consensus(1825)									

FIG. 80E

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TataminoSF162.opt

ATGGAGCCCGTGGACCCCGCCCTGGAGCCCTGGAGCACCACCCCGGCAGCCAGCCCCAA
GACCGCCTGGCACCACAACTGCTACCTGCAAGAAGTGCTGCTTCCACTGCCAAGGTGTGCTT
CATCACCAAGGGCCTGGGCATCAGCTACGGCCGCAAGAAAGCGCCGCCAGCGCCGC

FIG. 81
(SEQ ID NO:89)

Tat_Cys22_SF162

MEPVDPRLEPWKHPGSQPKTAGTNCYCKKCCFHCQVCFITKGLGISYGRKKRRRRAPPDSE
VHQVSLPKQPASQPQGDPTGPKESKKKVERETETDPVHZ

FIG. 82
(SEQ ID NO:90)

SEQUENCE LISTING

<110> Chiron Corporation

<120> IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION
OF VIRUS-LIKE PARTICLES

<130> 1621.100

<140>

<141>

<160> 90

<170> PatentIn Ver. 2.0

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<212> DNA

<213> Human immunodeficiency virus

<400> 1

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1509

<210> 2

<211> 1845

<212> DNA

<213> Human immunodeficiency virus

<400> 2

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<211> 4313

<212> DNA

<213> Human immunodeficiency virus

<400> 3

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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
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<400> 4

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<210> 5

<211> 1853

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
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<400> 5

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aaggaggggc acaccgccag gaactgccgc gcccccgca agaagggtg ctggcgctgc 1260
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atctggcctt cctacaaggg aaggccaggg aattttcttc agagcagacc agagccaaca 1380
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tgctgaccca gatcggctgc accctgaact tccccatcag ccccatcgag acggtgcccc 1800
tgaagctgaa gccgggggatg gacggcccca aggtcaagca gtggccctg taa 1853

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<210> 6

<211> 4319

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag-polymerase

<400> 6

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gccaccatgg gcgcccgcgc cagcgtgctg agcggcgggc agctggacaa gtgggagaag 60
atccgcctgc gccccggcgg caagaagaag tacaagctga agcacatcgt gtgggcccagc 120
cgcgagctgg agcgttcgc cgtgaacccc ggctgctgg agaccagcga gggctgccgc 180
cagatcctgg gccagctgca gccagcctg cagaccggca gcgaggagct gcgcagcctg 240
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gaggccctgg agaagatcga ggaggagcag aacaagtcca agaagaaggc ccagcaggcc 360
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gcagcgacct ggagatcggc cagcaccgca ccaagatcga ggagctgcgc cagcacctgc 2400
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acaaggaccc cctgtggaag ggccccgcca agctgctgtg gaagggcgag ggcgccgtgg 4200
tgatccagga caacagcgac atcaagggtg tgccccggcg caaggccaag atcatccgcg 4260
actacggcaa gcagatggcc ggcgacgact gcgtggccag ccgccaggac gaggactag 4319

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<210> 7

<211> 2031

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag/HCV-core fusion polypeptide

<400> 7

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gccaccatgg gcgcccgcgc cagcgtgctg agcggcggcg agctggacaa gtgggagaag 60
atccgcctgc gcccggcgcg caagaagaag tacaagctga agcacatcgt gtgggccagc 120
cgcgagctgg agcgcttcgc cgtgaacccc ggctgctgg agaccagcga gggctgccgc 180
cagatcctgg gccagctgca gccagcctg cagaccggca gcgaggagct gcgcagcctg 240
tacaacaccg tggccaccct gtactgcgtg caccagcgca tcgacgtcaa ggacaccaag 300
gaggccctgg agaagatcga ggaggagcag aacaagtcca agaagaaggc ccagcaggcc 360
gccgcccggc ccggcaccgg caacagcagc caggtgagcc agaactacc catcgtgcag 420
aacctgcagg gccagatggt gcaccaggcc atcagcccc gcacctgaa cgctgggtg 480
aagggtggtg aggagaaggc cttcagcccc gaggtgatcc ccatgttcag cgccctgagc 540
gagggcgcca cccccagga cctgaacacg atgttgaaca ccgtgggcgg ccaccaggcc 600
gccatgcaga tgctgaagga gaccatcaac gaggaggccg ccgagtggga ccgctgcac 660
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gccggcacca ccagcaccct gcaggagcag atcggctgga tgaccaaaa ccccccatc 780
cccgtgggcg agatctacaa gcggtggatc atcctgggcc tgaacaagat cgtgcggatg 840
tacagcccca ccagcatcct ggacatccgc cagggccccca aggagccctt ccgcgactac 900

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```

<210> 8

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag/HCV-Core fusion polypeptide

<400> 8

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atgggtgcga gagcgtcggg attaagcggg ggagaattag ataaatggga aaaaattcgg 60
ttaaggccag ggggaaagaa aaaatataag ttaaaacata tagtatgggc aagcaggagg 120
ctagaacgat tcgcagtcaa tcctggcctg ttagaaacat cagaaggctg cagacaaata 180
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cagggggcaa tggtagatca ggccatatca cctagaactt taaatgcatg ggtaaaagta 480
gtagaagaaa aggttttcag ccagaaagta ataccatgt tttcagcatt atcagaagga 540
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caaatgttaa aagagactat caatgaggaa gctgcagaat gggatagagt gcatccagtg 660
catgcagggc ctattgcacc aggccaaatg agagaaccaa ggggaagtga catagcagga 720
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ggtagacgtc agcctatccc caaggctcgt cggcccaggg gcaggacctg ggctcagccc 1740

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ggtaagggtca tcgataccct tacgtgcggc ttccgccgacc tcatggggta cataccgctc 1920
gtcggcgccc ctcttgagg cgctgccagg gccctggcgc atggcggtccg ggttctggaa 1980
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```

<210> 9

<211> 1268

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Gag
common region

<400> 9

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gccaccatgg gcgcccgcgc cagcgtgctg agcggcggcg agctggacaa gtgggagaag 60
atccgcctgc gcccgcggcg caagaagaag tacaagctga agcacatcgt gtgggccagc 120
cgcgagctgg agcgcttcgc cgtgaacccc ggcctgctgg agaccagcga gggctgccgc 180
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atgatgcagc gcggcaactt ccgcaaccag cggaagaccg tcaagtgtt caactgcggc 1200
aaggagggcc acaccgccag gaactgccgc gcccccgca agaagggtg ctggcgctgc 1260
ggccgcga 1268

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<210> 10

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: HIV-Gag
peptide p7G

<400> 10

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Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu
  1           5           10           15

```

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Glu Ala Ala Glu
      20

```

<210> 11

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer GAG5

<400> 11

aagaattcca tgggtgag agcgtaggta

30

<210> 12

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer
p55-SAL3

<400> 12

attcgtagc tgtgacgagg ggtcgtagc

30

<210> 13

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer
CORESAL5

<400> 13

atttgtagc gaatcctaaa cctcaaagaa aaac

34

<210> 14

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer 173CORE

<400> 14

tattggatcc taagagcaac caggaagggt c

31

<210> 15

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer MS65

<400> 15

cgaccatcat ggatgcagcg c

21

<210> 16

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer MS66

<400> 16

aggattcgtc gagtcgctgc tggggtcggt

30

<210> 17

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer XPANXNF

<400> 17

gcacgtgggc ccggcgctc tagagc

26

<210> 18

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer XPANXNR

<400> 18

gctctagagg cgccgggccc acgtgc

26

<210> 19

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: HIV p55 Gag
Major Homology Region

<400> 19

Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg
1 5 10 15Phe Tyr Lys Thr
20

<210> 20

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic p55
Gag Major Homology Region

<400> 20

gacatccgcc agggccccc aa ggagcccttc cgcgactacg tggaccgctt ctacaagacc 60

<210> 21

<211> 15

<212> PRT

<213> Human immunodeficiency virus

<400> 21

Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg
1 5 10 15

<210> 22

<211> 5

<212> PRT

<213> Human immunodeficiency virus

<400> 22

Lys Ala Lys Arg Arg
1 5

<210> 23

<211> 4

<212> PRT

<213> Human immunodeficiency virus

<400> 23

Arg Glu Lys Arg
1

<210> 24

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: aa of
mut7.SF162 cleavage site

<400> 24

Ala Pro Thr Lys Ala Ile Ser Ser Val Val Gln Ser Glu Lys Ser
1 5 10 15

<210> 25

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: aa of
mut8.SF162 cleavage site

<400> 25

Ala Pro Thr Ile Ala Ile Ser Ser Val Val Gln Ser Glu Lys Ser
1 5 10 15

<210> 26

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: aa of
mut.SF162 cleavage site

<400> 26

Ala	Pro	Thr	Lys	Ala	Lys	Arg	Arg	Val	Val	Gln	Arg	Glu	Lys	Ser
1				5					10					15

<210> 27

<211> 15

<212> PRT

<213> Human immunodeficiency virus

<220>

<223> Description of Artificial Sequence: aa of native
cleavage site in US4

<400> 27

Ala	Pro	Thr	Gln	Ala	Lys	Arg	Arg	Val	Val	Gln	Arg	Glu	Lys	Arg
1				5					10					15

<210> 28

<211> 5

<212> PRT

<213> Human immunodeficiency virus

<220>

<223> Description of Artificial Sequence: aa of second
cleavage site in US4

<400> 28

Gln	Ala	Lys	Arg	Arg
1				5

<210> 29

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: aa of mut.US4
cleavage site

<400> 29

Ala	Pro	Thr	Gln	Ala	Lys	Arg	Arg	Val	Val	Gln	Arg	Glu	Lys	Ser
1				5					10					15

<210> 30

<211> 1419

<212> DNA

<213> Human immunodeficiency virus

<400> 30

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<210> 31

<211> 1932

<212> DNA

<213> Human immunodeficiency virus

<400> 31

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<210> 32

<211> 2457

<212> DNA

<213> Human immunodeficiency virus

<400> 32

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<210> 33

<211> 1453

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp120.modSF162

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<210> 34

<211> 1387

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
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<400> 34

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<211> 1323

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<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

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<210> 37

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

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cccaccaagg ccaagcggcg cgtggtgcag cgcgagaagc gcgcctgac cctgggcgcc 1440

```



```

atgttcctgg gcttcctggg cgccgccggc agcaccatgg gcgcccgcag cctgaccctg 1500
accgtgcagg cccgccagct gctgagcggc atcgtgcagc agcagaacaa cctgctgcgc 1560
gccatcgagg cccagcagca cctgctgcag ctgaccgtgt ggggcatcaa gcagctgcag 1620
gcccgcgtgc tggccgtgga gcgctacctg aaggaccagc agctgctggg catctggggc 1680
tgcagcggca agctgatctg caccaccgcc gtgccctgga acgccagctg gagcaacaag 1740
agcctggacc agatctggaa caacatgacc tggatggagt gggagcgcga gatcgacaac 1800
tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggcc agcctgtgga actgggttcga catcagcaag 1920
tggctgtggt acatctaact cgag                                     1944

```

<210> 38

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gpl40.modSF162.delV1/V2

<400> 38

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggccctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgctg tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgccaccaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtggggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagc cctgccccaa ggtgagcttc 540
gagcccatcc ccatccacta ctgcgcccc ccgggcttcg ccatectgaa gtgcaacgac 600
aagaagttca acggcagcgg cccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
atccgccccg tggtagcac ccagctgctg ctgaacggca gcctggccga ggagggcgtg 720
gtgatccgca gcgagaactt caccgacaac gccaaagacca tcatcgtgca gctgaaggag 780
agcgtggaga tcaactgcac ccgccccaac aacaacaccc gcaagagcat caccatcggc 840
cccgggcgcg ccttctacgc caccggcgac atcatcggcg acatccgcca ggcccactgc 900
aacatcagcg gcgagaagtg gaacaacacc ctgaagcaga tcgtgaccaa gctgcaggcc 960
cagttcgcca acaagaccat cgtgttcaag cagagcagcg gcggcgaccc cgagatcgtg 1020
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agcacctgga acaacaccat cggccccaac aacaccaacg gcaccatcac cctgccctgc 1140
cgcataaagc agatcatcaa ccgctggcag gaggtgggca aggccatgta cgcccccccc 1200
atccgcggcc agatccgctg cagcagcaac atcaccggcc tgctgctgac ccgcgacggc 1260
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cccaccaagg ccaagcgccg cgtggtgcag cgcgagaagc gcgccgtgac cctggggcgcc 1440
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accgtgcagg cccgccagct gctgagcggc atcgtgcagc agcagaacaa cctgctgcgc 1560
gccatcgagg cccagcagca cctgctgcag ctgaccgtgt ggggcatcaa gcagctgcag 1620
gcccgcgtgc tggccgtgga gcgctacctg aaggaccagc agctgctggg catctggggc 1680
tgcagcggca agctgatctg caccaccgcc gtgccctgga acgccagctg gagcaacaag 1740
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggcc agcctgtgga actgggttcga catcagcaag 1920
tggctgtggt acatctaact cgag                                     1944

```

<210> 39

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modSF162

<400> 39

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgctgct ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcgggcag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gccctgttct acaagctgga cgtggtgccc 540
atcgacaacg acaacaccag ctacaagctg atcaactgca acaccagcgt gatcaccag 600
gcctgcccc aagtgagctt cgagcccatc cccatccact actgcgcccc cgccggcttc 660
gccatcctga agtgcaacga caagaagttc aacggcagcg gcccctgcac caacgtgagc 720
accgtgcagt gcacccacgg catccgcccc gtggtgagca cccagctgct gctgaacggc 780
agcctggcgg aggagggcgt ggtgatccgc agcgagaact tcaccgacaa cgccaagacc 840
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cgcaagagca tcaccatcgg ccccgccgc gccttctacg ccaccggcga catcatcggc 960
gacatccgcc aggccactg caacatcagc ggcgagaagt ggaacaacac cctgaagcag 1020
atcgtgacca agctgcaggc ccagttcggc aacaagacca tcgtgttcaa gcagagcagc 1080
ggcggcgacc ccgagatcgt gatgcacagc ttcaactgcg gcggcgagtt cttctactgc 1140
aacagcacc agctgttcaa cagcacctgg aacaacacca tcggccccaa caacaccaac 1200
ggcaccatca ccctgccctg ccgcatcaag cagatcatca accgctggca ggaggtgggc 1260
aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 1320
ctgctgctga cccgcgacgg cggcaaggag atcagcaaca ccaccgagat cttccgcccc 1380
ggcggcgggc acatgcgcga caactggcgc agcgagctgt acaagtacaa ggtggtgaag 1440
atcgagcccc tgggctgtgg ccccaaccaag gccaagcgcc gcgtggtgca gcgcgagaag 1500
agcgccgtga ccctgggccc catgttctct ggcttctctg gcgcgcggc cagcaccatg 1560
ggcgccccga gcctgaccct gaccgtgcag gcccgccagc tgctgagcgg catcgtgcag 1620
cagcagaaca acctgctgcg cgccatcag gcccagcagc acctgctgca gctgaccgtg 1680
tggggcatca agcagctgca ggcccgcgtg ctggccgtgg agcgctacct gaaggaccag 1740
cagctgctgg gcatctgggg ctgcagcggc aagctgatct gcaccaccgc cgtgccctgg 1800
aacgccagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

```

<210> 40

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modSF162.delV2

<400> 40

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgctgct ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcgggcag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagc cctgccccaa ggtgagcttc 540

```

```

gagcccatcc ccatccacta ctgcgcccc gccggcttcg ccatcctgaa gtgcaacgac 600
aagaagttca acggcagcgg cccctgcacc aacgtgagca ccgtgcagtg caccacaggc 660
atccgccccg tggtagacac ccagctgctg ctgaacggca gcctggccga ggagggcgtg 720
gtgatccgca gcgagaactt caccgacaac gccaaagacca tcatcgtgca gctgaaggag 780
agcgtggaga tcaactgcac ccgccccaac aacaacaccc gcaagagcat caccatcggc 840
cccgggccg ccttctacgc caccggcgac atcatcggcg acatccgcca ggcccactgc 900
aacatcagcg gcgagaagtg gaacaacacc ctgaagcaga tcgtgaccaa gctgcaggcc 960
cagttcggca acaagaccat cgtgttcaag cagagcagcg gcggcgaccc cgagatcgtg 1020
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agcacctgga acaacaccat cggccccaac aacaccaacg gcaccatcac cctgccctgc 1140
cgcacaaagc agatcatcaa ccgctggcag gaggtgggca aggccatgta cggccccccc 1200
atccgcggcc agatccgctg cagcagcaac atcaccggcc tgctgctgac ccgcgacggc 1260
ggcaaggaga tcagcaacac caccgagatc ttccgcccc gcggcgccga catgcgcgac 1320
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cccaccaagg ccaagcgccg cgtggtgcag cgcgagaaga gcgccgtgac cctgggcgcc 1440
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accgtgcagg cccgccagct gctgagcggc atcgtgcagc agcagaacaa cctgctgcgc 1560
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tgcagcggca agctgatctg caccaccgcc gtgccctgga acgccagctg gagcaacaag 1740
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag
1944

```

<210> 41

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modSF162.delV1/V2

<400> 41

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccggttgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgggcgc cggcaactgc cagaccagcg tgatcaccca ggcctgcccc 420
aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgcccgtt cgccatcctg 480
aagtgcacg acaagaagt caacggcagc ggcccctgca ccaacgtgag caccgtgcag 540
tgacccacg gcacccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
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caggcccact gcaacatcag cggcgagaag tggacaaca ccctgaagca gatcgtgacc 840
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tacgcccccc ccatccgcg ccagatccgc tgcagcagca acatcaccgg cctgctgctg 1140
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gacatgcgcg acaactggcg cagcgagctg tacaagtaca agtggtgaa gatcgagccc 1260
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accctgggcg ccatgttcct gggcttcctg ggcgcccgc gcagcaccat gggcgcccgc 1380
agcctgaccc tgaccgtgca ggcccggcag ctgctgagcg gcacgtgca gcagcagaac 1440

```

```

aacctgctgc gcgccatcga ggcccagcag cacctgctgc agctgaccgt gtggggcatc 1500
aagcagctgc aggcccgcgt gctggccgtg gagcgctacc tgaaggacca gcagctgctg 1560
ggcatctggg gctgcagcgg caagctgac tgcaccaccg cctgcccctg gaacgccagc 1620
tggagcaaca agagcctgga ccagatctgg aacaacatga cctggatgga gtgggagcgc 1680
gagatcgaca actacaccaa cctgatctac accctgatcg aggagagcca gaaccagcag 1740
gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
gacatcagca agtggctgtg gtacatctaa ctcgag 1836

```

<210> 42

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut7.modSF162

<400> 42

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacctt gcaactgcac aacctgaaga acgccaccaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gccctgttct acaagctgga cgtggtgccc 540
atcgacaacg acaacaccag ctacaagctg atcaactgca acaccagcgt gatcacccag 600
gcctgcccc aagtgagctt cgagcccatc cccatccact actgcgcccc cgccggcttc 660
gccatcctga agtgcaacga caagaagttc aacggcagcg gcccctgcac caacgtgagc 720
accgtgcagt gcacccacgg catccgcccc gtggtgagca cccagctgct gctgaacggc 780
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aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 1320
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aacgccagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

```

<210> 43

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut7.modSF162.delV2

<400> 43

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgctgct ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacct gcactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcgggcag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
gagcccatcc ccatccacta ctgcgcccc gccggcttcg ccctcctgaa gtgcaacgac 600
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aacatcagcg gcgagaagtg gaacaacacc ctgaagcaga tcgtgaccaa gctgcaggcc 960
cagttcggca acaagaccat cgtgttcaag cagagcagcg gcggcgaccc cgagatcgtg 1020
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agcacctgga acaacaccat cggccccaac aacaccaacg gcaccatcac cctgccctgc 1140
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cccaccaagg ccatcagcag cgtggtgcag agcgagaaga gcgccgtgac cctgggcgcc 1440
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accgtgcagg cccgccagct gctgagcggc atcgtgcagc agcagaacaa cctgctgcgc 1560
gccatcgagg cccagcagca cctgctgcag ctgaccgtgt ggggcatcaa gcagctgcag 1620
gcccgcgtgc tggccgtgga gcgctacct aaggaccagc agctgctggg catctggggc 1680
tgacgcggca agctgatctg caccaccgcc gtgccctgga acgccagctg gagcaacaag 1740
agcctggacc agatctggaa caacatgacc tggatggagt gggagcgcga gatcgacaac 1800
tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag 1944

```

<210> 44

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut7.modSF162.delV1/V2

<400> 44

```

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gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
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cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aagtgcacg acaagaagtt caacggcagc ggcccctgca ccaacgtgag caccgtgcag 540
tgacccacg gcatccgcc cgtggtgagc accagctgc tgctgaacgg cagcctggcc 600
gaggagggcg tggtagatccg cagcgagaac ttcaccgaca acgccaagac catcatcgtg 660

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cagctgaagg agagcgtgga gatcaactgc acccgcccca acaacaacac ccgcaagagc 720
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caggcccact gcaacatcag cggcgagaag tggacaaca ccctgaagca gatcgtgacc 840
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cccgagatcg tgatgcacag cttcaactgc ggcggcgagt tcttctactg caacagcacc 960
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gacatgcgcg acaactggcg cagcgagctg tacaagtaca aggtggtgaa gatcgagccc 1260
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agcctgaccc tgaccgtgca gggccgcccag ctgctgagcg gcatcgtgca gcagcagaac 1440
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aagcagctgc agggccgctg gctggccgtg gagcgctacc tgaaggacca gcagctgctg 1560
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tggagcaaca agagcctgga ccagatctgg aacaacatga cctggatgga gtgggagcgc 1680
gagatcgaca actacaccaa cctgatctac accctgatcg aggagagcca gaaccagcag 1740
gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
gacatcagca agtggctgtg gtacatctaa ctcgag 1836

```

<210> 45

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut8.modSF162

<400> 45

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cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgccaa ggcctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgccaccaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gccctgttct acaagctgga cgtggtgccc 540
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gcctgccccca aggtgagctt cgagcccatc cccatccact actgcgcccc cgccggcttc 660
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accgtgcagt gcacccacgg catccgcccc gtggtgagca ccagctgct gctgaacggc 780
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ggcggcccga gcctgaccct gaccgtgcag gcccggcagc tgctgagcgg catcgtgcag 1620
cagcagaaca acctgctgcg cgccatcgag gcccagcagc acctgctgca gctgaccgtg 1680

```

```

tggggcatca agcagctgca ggcccgcgtg ctggccgtgg agcgctacct gaaggaccag 1740
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aacgccagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

```

<210> 46

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut8.modSF162.delV2

<400> 46

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgccaa ggcctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgggccc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
gagcccatcc ccatccacta ctgcgcccc gccggcttcg ccacccgaa gtgcaacgac 600
aagaagttca acggcagcgg ccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
atccgccccg tggtagcac ccagctgctg ctgaacggca gcctggccga ggagggcgtg 720
gtgatccgca gcgagaactt caccgacaac gccaaagacca tcatcgtgca gctgaaggag 780
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cccggccgcg ccttctacgc caccggcgac atcatcggcg acatccgcca ggccactgc 900
aacatcagcg gcgagaagtg gaacaacacc ctgaagcaga tcgtgaccaa gctgcaggcc 960
cagttcggca acaagaccat cgtgttcaag cagagcagcg gcggcgaccc cgagatcgtg 1020
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agcacctgga acaacaccat cgccccaa aacaccaacg gcaccatcac cctgccctgc 1140
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag 1944

```

<210> 47

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut8.modSF162.delV1/V2

<400> 47

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gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcca ggcctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aagggtgagct tcgagcccat ccccatccac tactgcgccc ccgccggctt cgccatcctg 480
aagtgcacg acaagaagtt caacggcagc ggcccctgca ccaacgtgag caccgtgcag 540
tgcaccacg gcattccgcc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
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cagctgaagg agagcgtgga gatcaactgc acccgcccca acaacaacac ccgcaagagc 720
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accctgccc gccgcatcaa gcagatcatc aaccgctggc aggaggtggg caaggccatg 1080
tacgcccccc ccatccgcgc ccagatccgc tgcagcagca acatcacccg cctgctgctg 1140
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gacatgcgcg acaactggcg cagcgagctg tacaagtaca aggtggtgaa gatcgagccc 1260
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tggagcaaca agagcctgga ccagatctgg aacaacatga cctggatgga gtgggagcgc 1680
gagatcgaca actacaccaa cctgatctac accctgatcg aggagagcca gaaccagcag 1740
gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
gacatcagca agtggctgtg gtacatctaa ctcgag 1836

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<210> 48

<211> 2547

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp160.modSF162

<400> 48

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cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcca ggcctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcaactgcac aacctgaaga acgcccacaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtag gccctgttct acaagctgga cgtggtgccc 540
atcgacaacg acaacaccag ctacaagctg atcaactgca acaccagcgt gatcacccag 600
gcctgcccc aagtgagctt cgagcccatc cccatccact actgcgccc cgcgggcttc 660
gccatcctga agtgcaacga caagaagttc aacggcagcg gcccctgcac caacgtgagc 720
accgtgcagt gcacccacgg catccgcccc gtggtgagca cccagctgct gctgaacggc 780
agcctggcgc aggagggcgt ggtgatccgc agcgagaact tcaccgacaa cgccaagacc 840
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cgcaagagca tcaccatcgg ccccgccgc gccttctacg ccaccggcga catcatcggc 960
gacatccgcc aggcccatg caacatcagc ggcgagaagt ggaacaacac cctgaagcag 1020

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```

atcgtgacca agctgcaggc ccagttcggc aacaagacca tcgtgttcaa gcagagcagc 1080
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aacagcaccc agctgttcaa cagcacctgg aacaacacca tcggcccca caacaccaac 1200
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aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 1320
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gccgtgagcc tgttcgacgc catcgccatc gccgtggccg agggcaccga ccgcatcatc 2460
gaggtggccc agcgcacatcg ccgcgccttc ctgcacatcc cccgcgcgat ccgccagggc 2520
ttcgagcgcg ccctgctgta actcgag 2547

```

<210> 49

<211> 2466

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modSF162.delV2

<400> 49

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcaactgcac aacctgaaga acgcccacaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagc cctgccccaa ggtgagcttc 540
gagcccatcc ccattcacta ctgcgcccc gcccgtctcg ccattcctgaa gtgcaacgac 600
aagaagtcca acggcagcgg ccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
atccgccccg tggtagcac ccagctgctg ctgaacggca gcctggccga ggaggcgtg 720
gtgatccgca gcgagaactt caccgacaac gccaaagacca tcatcgtgca gctgaaggag 780
agcgtggaga tcaactgcac ccgcccacac aacaacaccc gcaagagcat caccatcggc 840
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atccgcgggc agatccgctg cagcagcaac atcaccggcc tgctgctgac ccgcgacggc 1260
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```

```

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aacctgctgc agtactggat ccaggagctg aagaacagcg ccgtgagcct gttcgacgcc 2340
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cgcgccttcc tgcacatccc ccgccgcac cgcagggtc tcgagcgcgc cctgctgtaa 2460
ctcgag

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<210> 50

<211> 2358

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modSF162.delV1/V2

<400> 50

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcctacgac 180
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cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgcccggctt cgccatcctg 480
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```

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```

<210> 51

<211> 1494

<212> DNA

<213> Human immunodeficiency virus

<400> 51

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```

<210> 52

<211> 2007

<212> DNA

<213> Human immunodeficiency virus

<400> 52

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ttatgggac aaagcctaaa gccatgtgta aaattaaccc cactctgtgt tactttaaat 300
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ccaattccca tacattattg tgccccggct ggttttgcca ttctaaagtg taaagataag 660
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agaccagtag tatcaactca actgctgtta aatggcagtc tagcagaaga agagatagta 780
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gaaattggca attatacagg cttaatatat aatttaattg aaatagcaca aaaccagcaa 1920
gaaaagaatg aacaagaatt attggaatta gacaagtggg caagtttgtg gaattggttt 1980
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```

<210> 53

<211> 2532

<212> DNA

<213> Human immunodeficiency virus

<400> 53

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gaatattctc tcttctataa acttgatgta gtaccaatag ataatgataa tgctagctat 540
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gctttactat aa 2532

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<210> 54

<211> 1599

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp120.modUS4

<400> 54

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tacaaggtgg tgcgcatcga gccctggggc gtggccccc cccaggccaa gcgcccgtg 1560
gtgcagcgcg agaagcgcta agatatcgga tcctctaga 1599

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<210> 55

<211> 1350

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp120.modUS4.del 128-194

<400> 55

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
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gtgcgcatcg agcccctggg cgtggccccc acccaggcca agcgcgcgct ggtgcagcgc 1320
gagaagcgct aagatatcgg atcctctaga                                     1350

```

<210> 56

<211> 2112

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp140.modUS4

<400> 56

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ggatcctcta ga                                     2112

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<210> 57

<211> 2112

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gpl40.mut.modUS4

<400> 57

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atcgagatcg cccagaacca gcaggagaag aacgagcagg agctgctgga gctggacaag 2040
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<210> 58

<211> 2181

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp140TM.modUS4

<400> 58

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<210> 59

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
 gp140.modUS4.delV1/V2

<400> 59

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gacaagtggg ccagcctgtg gaactggttc gacatcacca actggctgtg gtacatctaa 1800
gatatcggat cctctaga                                     1818

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<210> 60

<211> 2031

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.modUS4.delV2

<400> 60

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
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caggagaaga acgagcagga gctgctggag ctggacaagt gggccagcct gtggaactgg 1980
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<210> 61

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modUS4.delV1/V2

<400> 61

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gatatcggat cctctaga

1818

<210> 62

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.modUS4.del 128-194

<400> 62

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gatatcggat cctctaga 1818
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<210> 63

<211> 1863

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modUS4.del 128-194

<400> 63

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aga

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<210> 64

<211> 2634

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp160.modUS4

<400> 64

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agcgtgcgcg acaagggtgca gaaggagtac agcctgttct acaagctgga cgtggtgccc 600
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gctgccccca aggtgagctt cgagcccatc cccatccact actgcgccc cgccggcttc 720
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<210> 65

<211> 2538

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp160.modUS4.delV1

<400> 65

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cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
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aactgcagct tcaacatcac caccagcgtg cgcgacaagg tgcagaagga gtacagcctg 480
ttctacaagc tggacgtggg gccatcgac aacgacaacg ccagctaccg cctgatcaac 540
tgcaacacca gcgtgatcac ccaggcctgc cccaagggtga gcttcgagcc catccccatc 600
cactactgcg cccccgccgg ctctgccatc ctgaagtga aggacaagaa gttcaacggc 660
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cccaccagc ccaagcgccg cgtggtgcag cgcgagaagc gcgccgtggg cctgggcgcc 1500
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2538

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<210> 66

<211> 2553

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modUS4.delV2

<400> 66

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gaactgcacc gacaagctga ccggcagcac caacggcacc 420
aacagcacca gcggcaccaa cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgagggcgag atcaagaact gcagcttcaa catcggcgcc 540
ggcgcgctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 600
gagcccatcc ccattcacta ctgcgcccc gcccgttctg ccattcctgaa gtgcaaggac 660
aagaagtcca acggcaccgg cccctgcaag aacgtgagca ccgtgcagtg caccacggc 720
atccgccccg tggtagcac ccagctgctg ctgaacggca gcctggccga ggaggagatc 780
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aacatcagca aggccaactg gaccaacacc ctcgagcaga tcgtggagaa gctgcgcgag 1020
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cgcgccctgc tgtaagatat cggatcctct aga 2553

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<210> 67

<211> 2340

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modUS4.delV1/V2

<400> 67

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aacacgcgta agagcatcca catcgccccc ggccgcgcct tctacgccac cggcgacatc 720
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agccaggagc tgaagagcag cgccgtgagc ctgttcaacg ccaccgccat cgccgtggcc 2220
 gagggcaccg accgcatcat cgagatcgtg cagcgcatct tccgcgccgt gatccacatc 2280
 cccgcgcgca tccgccaggg cctggagcgc gccctgctgt aagatatcgg atcctctaga 2340

<210> 68

<211> 2385

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
 gp160.modUS4del 128-194

<400> 68

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 cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgccaa ggcttacaag 180
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 atcatcgaga tcgtgcagcg catcttccgc gccgtgatcc acatcccccg ccgcatccgc 2340
 cagggcctgg agcgcgcctt gctgtaagat atcggatcct ctaga 2385

<210> 69

<211> 144

<212> DNA

<213> Human immunodeficiency virus

<400> 69

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 aaggccatgt acgccccccc catccgcggc cagatcaagt gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 70

<211> 144

<212> DNA

<213> Human immunodeficiency virus

<400> 70

ggaactatca cactcccatg cagaataaaa caaattataa acaggtggca ggaagtagga 60
 aaagcaatgt atgcccctcc catcagagga caaattagat gctcatcaaa tattacagga 120
 ctgctattaa caagagatgg tggc 144

<210> 71

<211> 144

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Env
 US4 common region

<400> 71

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 aaggccatgt acgccccccc catccgcggc cagatcaagt gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 72

<211> 144

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Env
 SF162 common region

<400> 72

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 aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 73

<211> 4766

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
 gp160.modUS4.gag.modSF2

<400> 73

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4766

<210> 74

<211> 4689

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp160.modSF162.gag.modSF2

<400> 74

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<210> 75

<211> 4472

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

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<400> 75

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<210> 79

<211> 1865

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: GP2

<400> 79

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<210> 80

<211> 2305

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

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<400> 80

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<210> 82

<211> 2306

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

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<400> 82

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<211> 2300

<212> DNA

<213> Artificial Sequence

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<400> 83

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 <211> 306
 <212> DNA
 <213> Human immunodeficiency virus

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 cattag 306

<210> 86
 <211> 101
 <212> PRT
 <213> Human immunodeficiency virus

<400> 86
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 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Pro Asp Ser Glu Val
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ala Ser Gln Pro Gln Gly Asp
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<210> 87
 <211> 306
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<220>
 <223> Description of Artificial Sequence: tat.SF162.opt

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<210> 88
 <211> 306

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
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<400> 88

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<210> 89

<211> 168

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:
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<400> 89

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<210> 90

<211> 102

<212> PRT

<213> Artificial Sequence

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<400> 90

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          35             40             45
Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Pro Asp Ser Glu Val
          50             55             60
His Gln Val Ser Leu Pro Lys Gln Pro Ala Ser Gln Pro Gln Gly Asp
          65             70             75             80
Pro Thr Gly Pro Lys Glu Ser Lys Lys Lys Val Glu Arg Glu Thr Glu
          85             90             95
Thr Asp Pro Val His Glx
          100

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A61K 48/00

4560 Horton Street - R440, Emeryville, CA 94608 (US).
WALKER, Christopher; Chiron Corporation, 4560
Horton Street - R440, Emeryville, CA 94608 (US).

(21) International Application Number: PCT/US99/31245

(74) Agents: DOLLARD, Anne, S.; Chiron Corporation, In-
tellectual Property - R440, P.O. Box 8097, Emeryville, CA
94662-8097 et al. (US).

(22) International Filing Date:
30 December 1999 (30.12.1999)

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IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
UG, UZ, VN, YU, ZA, ZW.

(71) Applicant: CHIRON CORPORATION [US/US]; 4560
Horton Street, Emeryville, CA 94608 (US).

(84) Designated States (*regional*): ARIPO patent (GH, GM,
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(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors: BARNETT, Susan; Chiron Corporation, 4560
Horton Street - R440, Emeryville, CA 94608 (US). ZUR
MEGEDE, Jan; Chiron Corporation, 4560 Horton Street
- R440, Emeryville, CA 94608 (US). SRIVASTAVA,
Indresh; Chiron Corporation, 4560 Horton Street - R440,
Emeryville, CA 94608 (US). LIAN, Ying; Chiron Cor-
poration, 4560 Horton Street - R440, Emeryville, CA
94608 (US). HARTOG, Karin; Chiron Corporation, 4560
Horton Street - R440, Emeryville, CA 94608 (US). LIU,
Hong; Chiron Corporation, 4560 Horton Street - R440,
Emeryville, CA 94608 (US). GREER, Catherine; Chiron
Corporation, 4560 Horton Street - R440, Emeryville,
CA 94608 (US). SELBY, Mark; Chiron Corporation,

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ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

WO 00/39302 A3

(54) Title: IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION OF VIRUS-LIKE PARTICLES

(57) Abstract: The present invention relates to the efficient expression of HIV polypeptides in a variety of cell types, including, but not limited to, mammalian, insect, and plant cells. Synthetic expression cassettes encoding the HIV Gag-containing polypeptides are described, as are uses of the expression cassettes in applications including DNA immunization, generation of packaging cell lines, and production of Env-, tat- or Gag-containing proteins. The invention provides methods of producing Virus-Like Particles (VLPs), as well as, uses of the VLPs including, but not limited to, vehicles for the presentation of antigens and stimulation of immune

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/31245A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/49 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, STRAND, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 34640 A (MERCK & CO; SHIVER ET AL.) 13 August 1998 (1998-08-13) cited in the application claims 4,5; examples 3,4 ---	1-4
X	WO 97 31115 A (MERCK & CO; SHIVER ET AL.) 28 August 1997 (1997-08-28) page 54 nucleotides 856-995 example 11 ---	14,26, 29,32
X	WO 98 12207 A (GENERAL HOSPITAL CORPORATION) 26 March 1998 (1998-03-26) Figure 1 nucleotides 1315-1458 page 13 -page 21 --- -/--	14,26, 29,32

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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8 document member of the same patent family

Date of the actual completion of the international search

10 August 2000

Date of mailing of the international search report

22.08.00

Name and mailing address of the ISA,

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. +31 (0)70 300 9000

Authorized officer

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/31245

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 41397 A (OXFORD BIOMEDICA LTD; KINGSMAN ET AL.) 19 August 1999 (1999-08-19) SEQ ID NO:2 example 2	1-3
E	WO 00 15819 A (CHILDRENS MEDICAL CENTER;GRAY ET AL.) 23 March 2000 (2000-03-23) SEQ ID NO:4,pHDMH	1-3
A	--- SCHNEIDER R ET AL: "Inactivation of the human immunodeficiency virus type 1 inhibitory elements allows rev-independent expression of gag and gag/protease and particle formation" JOURNAL OF VIROLOGY, vol. 71, no. 7, July 1997 (1997-07), pages 4892-4903, XP002137891 AMERICAN SOCIETY FOR MICROBIOLOGY US cited in the application figure 1	1-13, 36-53
A	--- ANDRE S ET AL: "INCREASED IMMUNE RESPONSE ELICITED BY DNA VACCINATION WITH A SYNTHETIC GP120 SEQUENCE WITH OPTIMIZED CODON USAGE" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 72, no. 2, 1 February 1998 (1998-02-01), pages 1497-1503, XP002073767 ISSN: 0022-538X cited in the application the whole document	14,36-53
A	--- LU S ET AL: "IMMUNOGENICITY OF DNA VACCINES EXPRESSING HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ENVELOPE GLYCOPROTEIN WITH AND WITHOUT DELETIONS IN THE V1/2 AND V3 REGIONS" AIDS RESEARCH AND HUMAN RETROVIRUSES,US,MARY ANN LIEBERT, vol. 14, no. 2, 20 January 1998 (1998-01-20), pages 151-155, XP000907375 ISSN: 0889-2229 the whole document	15,17,20
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/31245

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>STAMATATOS L AND CHENG-MAYER C: "An envelope modification that renders a primary, neutralization-resistant clade B HIV-1 isolate highly susceptible to neutralization by sera from other clades" JOURNAL OF VIROLOGY, vol. 72, no. 10, October 1998 (1998-10), pages 7840-7845, XP002139602 AMERICAN SOCIETY FOR MICROBIOLOGY US the whole document</p> <p>-----</p>	15, 17, 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/31245

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 61-84 , 89 and 90 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-13, 57 and 58 (all completely); 36-56, 60-90 (all partly)

Expression cassette encoding an HIV gag polypeptide, vectors and cells comprising said cassette, uses thereof to produce polypeptides or virus-like particles, methods of treating a subject using said vectors.

2. Claims: 14-35 and 59 (all completely); 36-56 and 60-90 (all partly)

As subject 1 but limited to expression cassettes encoding HIV env polypeptide.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 99/31245

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